JOURNAL OF SURGICAL RESEARCH 16, 164-168 (1974)

Cholelithiasis in Mice: Effects of Different Chemicals Upon Formation and Prevention of Gallstones

SATINDRA K. GOSWAMI AND CHARLES F. FREY

CHOLECYSTITIS AND GALLSTONE formation in the mouse can be induced by a lithogenic diet containing 1% cholesterol and 0.5% cholic acid by weight [20]. Gallstones formed more rapidly and frequently in conventional than germ-free mice fed the lithogenic diet, although the development of cholecystitis was similar in onset and degree in both cases [12]. This finding and the inability to culture bacteria from the gallbladder wall or bile of conventional mice fed the lithogenic diet makes it unlikely that bacteria have any major role in gallstone formation in the mouse [13].

Spontaneous dissolution of gallstones in man has been reported [1, 16]. Diet-induced gallstones (proved by laparotomy) disappear in mice fed a chow diet for several months [8]. This kind of result has also been reported with other animal models [4, 10]. Prevention of gallstone formation in animals may be achieved by adding various chemicals to the diet [3, 5]. The purpose of the present study is to ascertain the effects of some chemicals on the formation and prevention of cholesterol gallstones in mice fed the lithogenic diet.

From Department of Surgery, Wayne County General Hospital, Eloise, Michigan and University of Michigan Medical Center, Ann Arbor, Michigan 48104.

The authors acknowledge the assistance given by Mrs. S. Goswami during the latter part of this experiment. This investigation was aided by a grant from the Medical Staff Research and Education Fund, Wayne County General Hospital.

Submitted for publication June 29, 1973.

MATERIALS AND METHODS

Three-week-old weanling CD-1 Swiss mice from Charles River Breeding Laboratory, Wilmington, MA were divided into nine groups on the basis of diet.

Group I. Five male and five female mice were fed the Notre Dame L-356 diet (General Biochemicals, Chagrin Falls, OH) for 8 weeks.

Group II. Ten mice, five male and five female, were fed the Notre Dame L-356 diet to which had been added 1% cholesterol (Nutritional Biochemicals Corp., Cleveland, OH) and 0:5% cholic acid (Nutritional Biochemicals Corp., Cleveland, OH) by weight.

Group III. Five male and five female mice were fed the lithogenic diet as in group II plus 3% *citrus pectin* (Sunkist Growers, Inc., Corona, CA).

Group IV. Ten mice, five male and five female, were fed 1% *egg lecithin* (Nutritional Biochemicals Corp.) along with the lithogenic diet as in group II.

Group V. Ten mice, five male and five female, were fed the lithogenic diet as in group II to which had been added 1% *n-octyl alcohol* (Matheson, Coleman and Bell, Norwood, OH).

Group VI. Ten mice, five male and five female, were fed the lithogenic diet as in group II along with 1% dodecyl sodium sulfate (Matheson, Coleman and Bell) for 4 weeks. After that, the dodecyl sodium sulfate concentration was changed to 0.5% prior to sacrifice.

Group VII. Ten mice, five male and five female, were fed 0.2% *neomycin sulfate* (Nutritional Biochemicals Corp.) together with the lithogenic diet as in group II for 4 weeks. After 4 weeks, the neomycin concentration was increased to 0.5% prior to sacrifice.

Group VIII. Five male and five female mice were fed the lithogenic diet as in group II plus 5% L-*ascorbic acid* (Nutritional Biochemicals Corp.).

Group IX. Ten mice, five male and five female, were fed the lithogenic diet as in group II together with 1.5% sulfaguanidine (Sigma Chemical Company, St. Louis, MO) prior to sacrifice.

All mice were sacrificed at the end of 8 weeks. After 24-hr fast the animals were anesthetized with ether, blood drawn from heart; liver and gallbladder were removed. Serum and liver were frozen immediately after collection. Liver was then lyophilized to a constant weight, ground to a powder, and kept in the refrigerator for further analysis. The gallbladder was opened on a glass slide and microscopic examination of the gallbladder contents was carried out under low power (1000×). The degree of cholesterol crystal formation was graded as no crystals, scattered crystals, and aggregates of crystals and gallstones according to Frey *et al.* [12]. The gallstones were balled aggregates large enough to be visible to the unaided eye.

Determination of cholesterol and lecithin was performed on pooled samples of serum and liver from the same group and sex. The cholesterol content of serum and liver was measured by the method of Glick *et al.* [14]. Lipid phosphorous was measured from a 2:1 chloroform; methanol (from Matheson, Coleman and Bell) extract by the method of Bartlett [2]. Phospholipid values were calculated by multiplying the phosphorous value by 25. All data were expressed as the mean.

RESULTS AND DISCUSSION

The effects of different diets on the formation of gallstones is shown in Table 1.

Table 1. Incidence of Gallstones in Mice Fed Different Diets

Diet	No Crystals	Scattered Crystals	Aggregates	Gallstones
Group I	10	0	0	0
Group II	1	0	1	8
Group III	0	1	1	8
Group IV	0	2	0	8
Group V	1	0	1	8
Group VI	9	1	0	0
Group VII	0	0	2	8
Group VIII	1	1	0	8
Group IX	9	1	0	0

Group I. Gallbladder contents of the mice tested did not contain crystals or stones and the liver was normal.

Group II. The livers were fatty; one male mouse did not have any crystals in the gallbladder; another male mouse had aggregates; the rest had gallstones.

Group III. Scattered crystals were seen in the gallbladder of one male and aggregates in one female mouse; the remainder contained gross stones. The livers were fatty in all of the mice.

Group IV. All livers were fatty; two male mice had scattered crystals in the gallbladder and the rest had stones.

Group V. No stones were found in one male mouse whose liver was slightly fatty. One female mouse had aggregates and the rest had gallstones. All livers were fatty.

Group VI. The livers were slightly fatty, gallbladder contents in one female mouse contained scattered crystals and the rest did not contain any stones. At the end of 4 weeks, the mice were in poor physical condition and failed to gain weight as compared to the mice in the rest of the groups. Consequently the concentration of dodecyl sodium sulfate was lowered from 1% to 0.5%. Four weeks after this change the mice had gained weight and their physical condition was comparable to mice in other groups.

Group VII. The livers were yellowish and fatty. The gallbladders of two male mice contained aggregates and the rest contained gallstones.

Group VIII. A normal liver was present in one male and the gallbladder of this mouse did not contain any crystals or stones; all other mice had fatty livers. The gallbladder of one female mouse contained scattered crystals. The gallbladders of all other male and female mice contained gallstones.

Group IX. The livers of all mice were normal. The gallbladder of one female mouse contained scattered crystals. The remainder were free of crystals or stones.

The addition of 1.5% sulfaguanidine (group IX) or dodecyl sodium sulfate (group VI) to the gallstone-producing diet (group II) prevented cholesterol stone formation. The condition of the liver, normal or fatty, could not be correlated with gallstone formation.

Serum and liver cholesterol levels (Table 2) were higher in mice forming gallstones than in non-stone formers. Female mice fed the sulfaguanidine had a higher serum cholesterol level than the males of the same group. In all other eight groups female mice had lower levels of serum and liver cholesterol compared to their male counterparts. The increment in liver cholesterol in mice fed the lithogenic diet was significantly higher in males than in females when compared with the control group. This finding confirms the observations reported by Caldwell and Levitsky [9]. The accumulation of high concentrations of cholesterol in the liver always accompanied gallstone formation in the mouse. Similar findings have been noticed with the prairie dog fed a high-cholesterol, egg yolk diet [7].

Serum lecithin concentration (Table 3) were higher in animals fed the normal (group I), dodecyl sodium sulfate (group VI) sulfaguanidine (group IX), and octyl alcohol (group V) diet. Female mice had a low serum lecithin in all nine groups except in those mice fed the *n*-octyl alcohol-fed diet (group V). The liver lecithin level (Table 3) was highest in the mice of group V and group VI and lowest level in group IX. High bile lecithin levels increase the solubility of cholesterol and delay stone formation. However, the octyl alcohol-fed mice (group V), in spite of a high serum and liver lecithin concentration, still formed gallstones. Serum phospholipid deficiency (mostly lecithin) has been noted in patients with gallstones [21].

Table 2. Serum and Liver Cholesterol Levels of Mice on Different Diets

Diet	Sex	Serum (mg/100 ml)		Liver (mg/100 g dry wt)	
Group I	M	119	112–125[a]	1860	1830–1903[a]
	F	91	80–97	1577	1476–1643
Group II	M	176	167–185	6020	5100–6571
	F	165	155–170	5896	5662–6041
Group III	M	202	195–215	6716	5918–7246
	F	158	150–168	6256	5638–6734
Group IV	M	176	165–182	5700	5124–5932
	F	160	150–165	5688	5018–5914
Group V	M	308	295–315	8588	8344–8616
	F	290	280–300	7356	6853–7838
Group VI	M	147	137–150	2815	2596–3161
	F	174	162–182	4864	4316–5196
Group VII	M	144	138–150	6747	6260–7377
	F	139	128–145	6568	6134–6913
Group VIII	M	189	185–195	7499	6890–7860
	F	161	150–167	7124	6943–7903
Group IX	M	158	155–165	2898	2772–2945
	F	205	197–208	4336	3930–4713

[a] Range of cholesterol concentrations.

Table 3. Mean Lecithin Concentrations of Serum and Liver of Mice on Different Diets

Diet	Sex	Serum (mg/100 ml)		Liver (mg/100 g, dry wt.)	
Group I	M	225	217–230[a]	3600	3512–3855[a]
	F	185	177–192	4507	4285–4660
Group II	M	217	196–220	2892	2513–2987
	F	166	157–170	3006	2866–3247
Group III	M	209	198–213	2025	1873–2219
	F	164	159–168	2256	1916–2388
Group IV	M	173	163–178	3522	3374–3718
	F	146	139–153	4140	4019–4398
Group V	M	235	225–241	4710	4538–4912
	F	267	261–274	4725	4550–4898
Group VI	M	239	233–243	4064	3941–4080
	F	215	210–220	3707	3646–3868
Group VII	M	157	146–164	2634	2421–2828
	F	120	111–125	2932	2730–4142
Group VIII	M	214	202–220	2217	2158–2332
	F	171	161–179	3327	3263–3423
Group IX	M	242	232–252	2604	2572–2708
	F	232	220–239	3301	3185–3412

[a] Range of lecithin concentrations.

Citrus pectin given orally prevented gallstone formation and caused gallstone dissolution in rabbits [5]. But in our mice, pectin had no effect in the prevention of gallstones. Pectin given orally has been found to increase the fecal excretion of cholesterol and lower the serum cholesterol in man fed a typical American diet [15]. But in our experiment pectin along with 1% cholesterol and 0.5% cholic acid did not lower the serum cholesterol level. Staub *et al.* found an elevation of serum cholesterol in rats fed a high carbohydrate diet containing pectin [19].

The ability to increase biliary phospholipid by feeding lecithin suggests that dietary modification may be important in the prevention and treatment of cholelithiasis [22]. However, stone dissolution was not detected when lecithin was added to the ration of rabbits fed a lithogenic diet [6], as in our mice, fed lecithin.

Large doses of L-ascorbic acid did not have any effect on the prevention of gallstones. *N*-octyl alcohol, which is known to block the absorption of cholate in low concentrations and assist absorption of cholate in high concentrations, was thought to have an effect on dispersing cholesterol [18]. However, feeding *n*-octyl alcohol along with the lithogenic diet did not prevent gallstone formation although accompanied by a high lecithin level in both serum and liver. Dodecyl sodium sulfate prevented the formation of gallstones in mice fed a lithogenic diet. This alkyl surfactant might have increased the solubility of cholesterol and reduced the incidence of gallstone formation.

Oral administration of large doses of neomycin prevents gallstone formation in the rabbit [5, 17]. The action of neomycin is thought to be due to the modification of the anaerobic intestinal micro flora. Sulfaguanidine added to the diet of mice increases the fecal excretion of cholesterol although bile salt and fatty acid excretions are not increased [11]. Neomycin did not prevent the gallstone formation in our mice whereas sulfaguanidine did. Thus, the antimicrobial effect of the latter substance was not responsible for the prevention of gallstones. Sulfaguanidine lowered liver cholesterol levels in our mice. Eyssen *et al.* have found lower liver cholesterol values in both germ-free and conventional mice fed sulfaguanidine and concluded that the cholesterol-lowering effect of sulfaguanidine was independent of its antimicrobial effect [11].

SUMMARY

Prevention of gallstones induced in mice by 1% cholesterol and 0.5% cholic acid (lithogenic diet) for 8 weeks was obtained by simultaneously feeding sulfaguanidine (1.5%) and dodecyl sodium sulfate (0.5–1.0%) along with the lithogenic diet. Citrus pectin (3%), egg lecithin (1%), *n*-octyl alcohol (1%), neomycin sulfate (0.2–0.5%) and L-ascorbic acid (5%) added to the lithogenic diet did not prevent gallstone formation. The condition of the liver, fatty or normal, in the experiment could not be correlated with the stone formation. Lower serum and liver cholesterol levels and an elevation of lecithin concentrations in serum was noticed in mice fed the sulfaguanidine and dodecyl sodium sulfate diet.

REFERENCES

1. Arcomano, J. P., Schwinger, H. N., and DeAngelis, J. The spontaneous disappearance of gallstones. *Am. J. Roentgenol. Radium Ther. Nucl. Med.* **99**:637, 1967.
2. Bartlett, G. R. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**:466, 1959.
3. Besancon, F., Marche, C., Barret, C., and Parrot, J. Pharmacology of gallbladder lithiasis. Preventive and curvative effects of various drugs in mice. Therapie **25**:463, 1970 (from *Chem. Abstr.* **73**:54486 p. 1970).
4. Borgman, R. F., and Haselden, F. H. Cholelithiasis in rabbits: Effects of diet upon formation and dissolution of gallstones. *Am. J. Vet. Res.* **29**:1287, 1968.
5. Borgman, R. F., and Haselden, F. H. Cholelithiasis in rabbits: Effects of several treatments on formation and dissolution of gallstones. *Am. J. Vet. Res.* **30**:1979, 1969.
6. Borgman, R. F., and Haselden, F. H. Cholelithiasis in rabbits: Effects of bile constituents and hormones on dissolution of gallstones. *Am. J. Vet. Res.* **30**:107, 1969.
7. Brenneman, D. E., Connor, W. E., Forker, E. L., and DenBesten, L.: The formation of abnormal bile and cholesterol gallstones from dietary cholesterol in the prairie dog. *J. Clin. Invest.* **51**:1495, 1972.
8. Caldwell, F. T., Levitsky, K., and Rosenberg, B. Dietary induction and dissolution of gallstones in mice. *JAMA* **188**:437, 1964.
9. Caldwell, F. T., and Levitsky, K. The gallbladder and gallstone formation. *Ann. Surg.* **166**:753, 1967.
10. Dam, H., and Christensen, F. Alimentary production of gallstones in hamsters. *Acta Pathol. Microbiol. Scand.* **30**:236. 1952.
11. Eyssen, H. J., Vanden Bosch, J. F., and Janssen, G. A. Cholesterol-lowering effect of sulfaguanidine in mice and rats. *Advan. Exp. Med. Biol.* **4**:549, 1969.
12. Frey, C. F., Thorpe, C., and Abrams, G. Gallstone formation in the germ-free mouse. *Am. J. Surg.* **115**:75, 1968.
13. Frey, C. F., and Freter, R. Gallstone formation in the conventional mouse. The role of bacteria. *Am. J. Surg.* **116**:868, 1968.
14. Glick, D., Fell, B. F., and Sjolin, K. E. Spectro photometric determination of nanogram amounts of total cholesterol in microgram quantities of tissue or microliter volumes of serum. *Anal. Chem.* **36**:1119, 1964.
15. Lopez, S. A., Hopson, J., and Krehl, W. A. Effect of dietary pectin on plasma and fecal lipids. *Fed. Proc.* **27**:485, 1968.
16. Margulies, S. E., Brogdon, B. G., Sindler, R. A., and Perilla, F. R. Disappearing gallstones. *Brit. J. Radiol.* **40**:549, 1967.
17. Mosbach, E. H. Effect of drugs on bile acid metabolism. *Advan. Exp. Med. Biol.* **4**:421, 1969.
18. Mufson, D. Investigations of surface properties of cholesterol particles in aqueous dispersions. Thesis dissertation for Ph.D. in the University of Michigan, 1968.
19. Staub, H. W., and Thiessen, R., Jr. Effect of dietary pectin on serum cholesterol in rats. *Fed. Proc.* **27**:221, 1968.
20. Tepperman, J., Caldwell, F. T., and Tepperman, H. M. Induction of gallstones in mice by feeding a cholesterol–cholic acid-containing diet. *Am. J. Physiol.* **206**:628, 1964.
21. Tompkins, R. K., Cornwell, D. G., Spreche, H. W., and Zollinger, R. W. Role of biliary phospholipids in the formation of human gallstones. *Surg. Forum* **18**:411, 1967.
22. Tompkins, R. K., Burke, L., Zollinger, R. M., and Cornwell, D. G. Elevation of phospholipid concentrations in human bile by feeding lecithin. *Fed. Proc.* **27**:573, 1968.