Dietary Bile Acids and Lipid Metabolism. III. Effects of Lithocholic Acid in Mammalian Species. (28890)

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Reports from this laboratory have shown dietary lithocholic acid to produce the ductular cell reaction in the liver of the chicken (1,2,3). Others have substantiated this observation(4,5). This bile acid has also been shown to elevate plasma cholesterol and lipid phosphorus levels in the chick(3,4,6).

The ductular cell reaction in the chicken did not differ appreciably from that reported in man and experimental animals for a variety of natural and experimental liver diseases(1,2,3,7). The ductular cell reaction consisted of proliferation of bile ductules and ducts, infiltration of inflammatory cells, and fibrosis. This lesion has been reportedly induced in the rabbit as a result of lithocholic acid ingestion(8,9). The influence of dietary lithocholic acid on plasma and liver lipids of mice and rats has been studied(10-13); however, the effect on the microscopic structure of the liver has not been reported. Beher *et al*(14) have reported that lithocholic acid was toxic and produced a bile duct hyperplasia when fed to hamsters at a level of 1% of the diet.

This report presents data on plasma and liver lipids, and on the microscopic structure of the liver of several mammalian species fed lithocholic acid.

Experimental. Young rabbits, guinea pigs, hamsters and mice were fed a stock diet which had been ground to a powder and in which had been incorporated 0, 0.25 or 0.50% lithocholic acid. A group of weanling rats was fed a 20% casein purified diet to which had been added 0.50% lithocholic acid. The animals received the diets for 3 weeks. Body weight and food consumption were determined at weekly intervals. All animals were housed in cages having raised wire floors. Room temperature (70°) and relative humidity (50%) were kept constant.

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At the end of the experimental period, blood was drawn by cardiac puncture while the animals were under ether anesthesia. All animals were necropsied, the liver excised, blotted free of excess blood and weighed. A portion of the liver was fixed in 10% neutral buffered formalin for histologic examination. The remainder of the liver was frozen and stored at −20°C until analyzed.

Plasma and liver were analyzed for cholesterol and lipid phosphorus and the liver was analyzed for total lipids as previously described(3). Liver sections were prepared as previously described(2).

The data were statistically evaluated by means of the "t" test.

Results. Gross and biochemical findings. The data for body weight, liver size and plasma and liver lipids are presented in Table I. There were no significant changes in body weight as a result of lithocholic acid feeding except in the case of the rabbit. The lower body weight observed for the rabbits fed lithocholic acid can undoubtedly be attributed to the appetite-depressing effect of lithocholic acid. These animals consumed only an average of 247 g of diet per animal throughout the 3-week experimental period as compared to an average of 3105 g for the control animals. Plasma cholesterol and lipid phosphorus levels were significantly changed in the rabbit and mouse studies only. The plasma cholesterol level was increased in the rabbits fed lithocholic acid and the lipid phosphorus livel of mice fed the highest level of the bile acid was also elevated. Liver size was increased in the guinea pigs and mice fed the 0.5% level of lithocholic acid only; the bile acid did not influence liver size of the other species studied. Liver cholesterol levels were not altered by lithocholic acid feeding in the guinea pig, hamster or rat studies. In the rabbit, lithocholic acid increased liver cholesterol levels, while in the

TABLE I. Body Weight, Plasma and Liver Lipids of Rabbits, Guinea Pigs, Hamsters, Mice and Rats Fed Diets With or Without Added Lithocholic Acid.

Dietary lithocholic acid, % of diet	Final body wt, g	Plasma: Cholesterol, mg/100 ml	Plasma: Lipid phosphorus, mg/100 ml	Liver*: Wt, % body wt	Liver*: Fat, %	Liver*: Cholesterol, mg/g	Liver*: Lipid phosphorus, mg/g
Rabbit							
0 (3)†	2858 ±144‡	94 ± 39	24.6 ± 1.3	3.2 ± .4	5.68 ±.04	4.79 ±.47	.97 ±.13
.25 (3)	1650 ±115	606 ±165	34.1 ± 6.7	2.6 ± .4	4.77 ±.21	7.63 ±.86	.88 ±.17
Guinea pig							
0 (6)	480 ± 49	32 ± 9	19.1 ± 1.9	4.7 ± .5	4.17 ±.28	2.86 ±.23	1.02 ±.09
.25 (7)	443 ± 65	38 ± 9	18.8 ± 1.2	4.8 ± .6	4.13 ±.40	3.08 ±.47	.93 ±.10
.50 (7)	405 ± 91	30 ± 9	18.7 ± 1.9	5.7 ± .6	3.29 ±.57	2.92 ±.54	.73 ±.12
Hamster							
0 (10)	91 ± 14	156 ± 26	—	4.4 ± .5	4.79 ±.82	4.04 ±.96	.89 ±.17
.25 (10)	93 ± 11	173 ± 27	—	4.6 ± .4	4.76 ±.55	3.87 ±.46	.83 ±.19
.50 (10)	90 ± 7	159 ± 31	—	4.5 ± .5	5.00 ±.60	4.06 ±.57	.69 ±.14
Mouse							
0 (10)	26 ± 2	142 ± 20	26.5 ± 5.5	5.6 ± .5	5.62 ±.65	5.44 ±.19	.83 ±.11
.25 (10)	25 ± 4	149 ± 27	30.6 ± 8.7	5.8 ± .4	5.38 ±.61	5.30 ±.23	.86 ±.14
.50 (10)	25 ± 4	140 ± 28	33.8 ± 5.4	9.5 ±3.5	4.32 ±.86	4.72 ±.50	.68 ±.08
Rat							
.50 (6)	212 ± 7	110 ± 8	7.4 ± .2	4.8 ± .1	4.54 ±.15	3.33 ±.23	.95 ±.02

* All liver values expressed on wet weight basis. † Values in parentheses represent No. of animals.
‡ Mean ± standard deviation.

mouse liver cholesterol levels were decreased at the highest level of lithocholic acid fed. Liver lipid phosphorus levels were significantly reduced by the highest level of lithocholic acid fed in the guinea pig, hamster and mouse studies. In the rat and rabbit, lithocholic acid did not affect liver lipid phosphorus levels.

Microscopic findings. The ductular cell reaction was observed in the liver of all species fed lithocholic acid. However, only 3 of the 6 rats fed lithocholic acid developed this lesion. In the rabbit and hamster, 0.25% dietary lithocholic acid resulted in the reaction; however, a level of 0.50% was required to induce this change in the mouse, guinea pig and rat. Increasing the level to 0.50% in the hamster did not increase the extent of the reaction. In the mouse, guinea pig and rat, the extent of the reaction was minimal, and varied between individual animals. It consisted of a slight increase in ductular cells with minimal ductule formation in the periportal tissues (Fig. 1). Polymorphonuclear leucocytes were intermingled with the proliferating ductular tissue. The ductular cell reaction was most extensive in the rabbit, where proliferating ductular cells, ductules and ducts replaced much of the normal hepatic parenchyma, disrupting the normal lobular pattern (Fig. 2, 3). These newly formed tissues were supported by reticulum and collagenous connective tissue fibers, and there was a slight infiltration with polymorphonuclear and mononuclear inflammatory cells. In the hamster, the extent of the reaction was between that observed in the guinea pig and mouse, and the rabbit.

Focal areas of coagulation necrosis were present in one rabbit and several of the livers of the other species, including one mouse and one guinea pig fed 0.25% lithocholic acid in which the ductular cell reaction was not observed (Fig. 4). Similar necrotic foci were not observed in any of the control livers. In several livers of each species in which the ductular cell reaction was present, these necrotic areas were not observed. The necrosis was of recent origin and was associated with only a minimal inflammatory cell reaction and no fibrosis. In all of the rabbits there was a slight fatty degeneration in the hepatocytes adjacent to the proliferating ductular tissues. In 2 rabbits there was inspissated

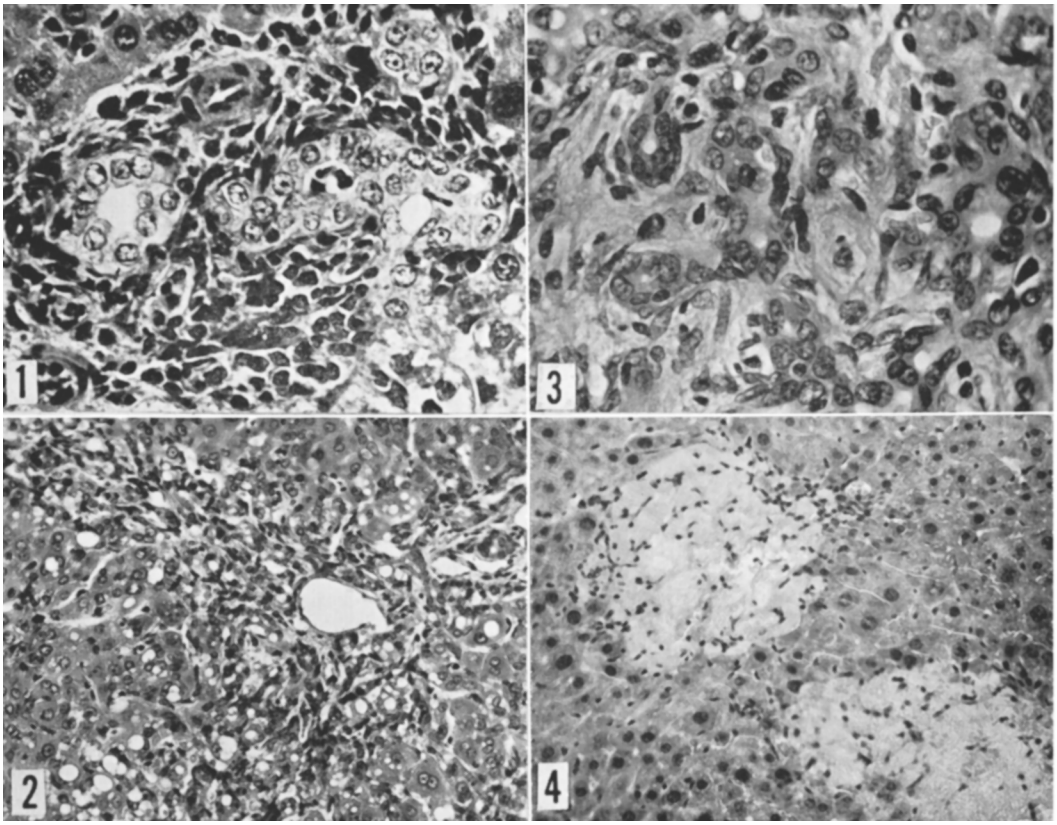

FIG. 1. Proliferated ductule cells in a rat liver 3 weeks after being placed on a synthetic diet containing 0.50% lithocholic acid. Most of the cells are not clearly recognizable as ductule epithelium, and obvious tubules have not been formed. Hematoxylin and eosin (H&E) stain × 375.

FIG. 2. Marked proliferation of ductule cells, many of which have formed tubules in a rabbit liver 3 weeks after being placed on a diet containing 0.25% lithocholic acid. There is fatty degeneration of the hepatocytes adjacent to the proliferated ductules. H&E stain × 175.

FIG. 3. Proliferated ductule cells, some of which have formed tubules in the liver of a rabbit fed 0.25% lithocholic acid. Collagenous connective tissue is present between ductule cells. H&E stain × 375.

FIG. 4. Focal areas of coagulation necrosis in liver of a mouse fed lithocholic acid for 3 weeks. H&E stain × 175.

bile within pre-existing and some of the newly formed bile ducts.

Unusual deposits of diastase resistant periodic acid-Schiff positive material were not observed in any of the livers. In the 3 rabbits with the ductular cell reaction there was an increase in stainable iron. This was primarily located at the junction of the proliferating ductules and the normal hepatic parenchyma, and appeared to be present in both Kupffer cells and hepatocytes. Hemosiderin was not seen in these areas in the hematoxylin and eosin stained sections.

Discussion. The observed effects of dietary lithocholic acid in the rabbit are in general agreement with the reports of Holsti(8) and Stolk(9). The lack of effect of the lowest level of lithocholic acid fed to mice and the relative ineffectiveness of this acid in the diet of rats is in agreement with the report of Eyssen *et al*(15). It is of interest to note that these authors, while finding no liver abnormalities in rats fed a lithocholic acid-supplemented complete diet, did observe marked bile duct proliferation in rats fed a protein-deficient, lithocholic acid-supplemented diet.

Lithocholic acid, when fed to mice, guinea pigs and hamsters, did not markedly alter the

various biochemical parameters studied. This observation is in contrast to its effect in the chick(3) and rabbit; however, lithocholic acid did, as in these 2 species, induce the ductular cell reaction.

These studies indicate that dietary lithocholic acid induces the ductular cell reaction in a variety of mammalian species in addition to the chicken, and adds this bile acid to the growing list of chemical agents capable of producing this change in the liver. The mechanism by which biliary proliferation is produced is not known; however, it is suggested that lithocholic acid is a primary growth stimulant to the biliary epithelium. It does not appear that the change is induced through damage to hepatocytes, as changes in these cells were not evident in all livers in which the ductular cell reaction was observed. Also, it has been demonstrated that the lesion is dependent on the continued presence of lithocholic acid in the diet. Removal of lithocholic acid from the diet results in complete reversal of the lesion(2). However, the discrete foci of necrosis observed in many of the livers indicate that lithocholic acid is capable of damaging hepatocytes. Cellular changes not discernible by light microscopy may have been present in those livers in which necrosis was not observed.

The production of the ductular cell reaction by lithocholic acid suggests the interesting hypothesis that this tissue change, as it occurs in natural and experimental hepatic diseases, may be the result of an abnormality of bile acid metabolism by the hepatocytes.

Summary. Lithocholic acid fed to rabbits, mice, guinea pigs, hamsters and rats has been shown to elevate plasma and liver cholesterol in the rabbit, lower plasma lipid phosphorus and liver cholesterol in the mouse, and lower liver lipid phosphorus levels in the guinea pig, hamster and mouse. In all species this bile acid induced a proliferation of bile ducts (ductular cell reaction), being most severe in the rabbit and hamster. The mechanism by which these changes were produced was not determined.

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