Dietary Bile Acids and Lipid Metabolism II. The Ductular Cell Reaction Induced by Lithocholic Acid.* (28300)

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Proliferation of bile ductules occurs in a number of spontaneous and experimentally induced hepatic diseases in man and animals. The proliferation, generally associated with the infiltration of inflammatory cells, has been designated as the "ductular cell reaction"(1). In man, it is a frequent lesion in obstructive jaundice, post necrotic cirrhosis, acute and subacute viral hepatitis, and acute and subacute alcoholic injury(1,2). In experimental animals, it is an especially prominent finding in ethionine, butter yellow, thioacetamine, senechio, and dimethylnitrosamine intoxications and avitaminosis A(1,3). The reaction has also been observed in rabbits following long term ingestion of desiccated bile(4). The active agent of bile has been shown to be lithocholic acid(5,6). In studies to determine the influence of dietary bile acids on cholesterol metabolism in chicken, we observed a marked increase in size of the liver in chickens fed lithocholic acid. Microscopic examination revealed the increase in liver size to be the result of the ductular cell reaction.

Materials and methods. A total of 139 chickens from 2 experiments were fed a basal diet described in Table I. At the expense of glucose, bile acids and/or cholesterol were added to the basal diet at a level of 0.2% and 2%, respectively, as depicted in Table II. The chickens were fed the experimental diets for the time periods indicated in Table II, then all of the birds except 32 (8 each from groups 9, 10, 11 and 12) were sacrificed. The remaining chickens at this time were fed the basal diet without additives for an additional 3 weeks prior to sacrifice.

All of the chickens were necropsied immediately after sacrifice. The liver was weighed and thin slices of liver, gall bladder, heart, aorta, lung, spleen, small intestine and kidney were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned at 5 μ in thickness, and stained with Harris' hematoxylin and eosin. Duplicate tissue sections of liver were also stained by Masson's trichrome, Gomori's iron and Giemsa's stains, and subjected to the periodic acid-Schiff (PAS) reaction preceded by diastase digestion.

Results. Gross findings. The liver was the only organ in which gross alteration was observed. In the birds fed lithocholic acid alone, or in combination with cholic acid or cholesterol (Groups 3, 4, 7, 11), the liver was enlarged up to 3.5 times that of the control chickens (Table II). In birds fed all 3 of the above additives (Group 8) there was no significant increase in liver weight. The surface of the enlarged livers was slightly nodular.

Microscopic findings. No lesions were observed in the control birds or the chickens fed cholic acid, deoxycholic acid or cholesterol alone or in combination (Groups 1, 2, 5, 7, 9, 10, 12). In the chickens fed lithocholic acid alone, or in combination with cholic acid or cholesterol (Groups 3, 4, 7, 11), there was a marked periportal proliferation of bile ductules and interlobular bile ducts throughout the liver (Fig. 1, 2). The newly formed biliary tissue existed as patent

TABLE I. Composition of Basal Diet.

Component	g/100 g diet
Glucose	54.99
Assay protein C-1	20.40
Salt mix	5.31
Corn oil	5.00
Non-nutritive fiber	4.00
Glycine	.40
DL-methionine	.30
Vitamin mix	.40
Choline chloride	.20

* The results of these studies with regard to growth and plasma and liver lipids will be reported separately.

TABLE II. Additives to Basal Diet and Liver Weight.*

Group	No. chickens	Weeks on diet	Additive	Liver wt†	
1	11	8	None	2.74 ± .39	
2	9	8	Cholic acid	2.57 ± .23	
3	8	8	Lithocholic acid	6.45 ± 1.44	
4	6	8	Cholic acid + lithocholic acid	8.16 ± 1.76	
5	11	8	Cholesterol	3.03 ± .26	
6	11	8	Cholic acid + cholesterol	3.52 ± .28	
7	7	8	Lithocholic acid + cholesterol	11.08 ± 4.91	
8	12	8	Lithocholic acid + cholic acid + cholesterol	3.69 ± .33	
9	16	3	None	2.2 ± .2	2.6 ± .4‡
10	16	3	Cholic acid	2.1 ± .3	2.6 ± .4‡
11	16	3	Lithocholic acid	8.5 ± 2.0	3.2 ± .3‡
12	16	3	Deoxycholic acid	2.2 ± .3	2.6 ± .3‡

* Bile acids and cholesterol were added at a level of 0.2% and 2% of the diet respectively, at the expense of glucose.

† Mean liver weight expressed at % total body wt ± standard deviation.

‡ % liver weight after returning the birds to the basal diet for an additional 3 weeks; see Materials and Methods.

tubules and solid islands and cords of cells without obvious lumens (Fig. 3). These structures extended in finger-like projections from the enlarged portal tracts into the hepatic lobules. In some cases the proliferated bile ductules extended and joined with similar proliferated bile ductules originating from other hepatic triads. The proliferated bile ductules and cells were supported by mature collagenous connective tissue which was in greater abundance than that which surrounds bile ducts in the normal liver. Numerous polymorphonuclear leucocytes infiltrated and surrounded the proliferated bile ductules. Small collections of lymphocytes were occasionally seen, but similar collections were seen in the livers of the control chickens. An occasional mitotic figure was observed in the hyperplastic epithelial cells. Large bile ducts, and the gall bladders appeared normal. Bizzare hepatocytes were not observed, nor were mitotic figures seen in hepatocytes. However, focal hepatic necrosis was observed in some of the affected livers. No unusual nonglycogenic PAS positive staining materials were observed in the abnormal livers. Minimal to extensive deposits of iron positive material were present in the livers of birds from all groups. There did not appear to be an increase in stainable iron in the abnormal livers.

The extent of the ductular cell reaction was approximately the same whether lithocholic acid was fed for 3 or 8 weeks. However, the reaction was more extensive in chickens fed lithocholic acid in combination with cholic acid or cholesterol than in those receiving lithocholic acid alone. In the chickens fed the combination of lithocholic acid, cholic acid and cholesterol, the reaction was absent to minimal in 10 birds and in 2 birds of the degree as seen when lithocholic acid was fed alone.

In the 8 chickens fed lithocholic acid for 3 weeks and then returned to the basal diet for 3 weeks prior to sacrifice, the ductular cell reaction was minimal. The portal triads were more cellular than normal, and short projections of cells extended from them into the hepatic lobules. These cells resembled fibroblasts, but were interpreted as ductular cells (interstitial cells, oval cells).

Discussion. The ductular cell reaction as seen in man and experimental animals consists of the proliferation of bile ductules and epithelial cells not forming obvious tubules with lumina. These latter cells have been called interstitial cells(1), oval cells(7), and more preferably ductular cells(8,9,10). Electron microscopic observations support the concept that these cells are derived from ductular and ductal epithelial cells(2). In addition to the proliferation of ductular cells, accumulation of inflammatory cells is a feature of the ductular cell reaction(9). The reaction in the chicken, as described here,

has the same characteristics as that which occurs in other experimental animals and man. Although the ductular cell reaction is known to occur in several diseases and experimental intoxications, the pathogenesis is not entirely understood. It has been suggested by Popper and Schaffner(8,11) that the reaction may be the result of excretion of an irritating material into the ductules and periductular tissue which serves as a stimulant for ductular proliferation and incites the inflammatory cell reaction and periductular fibrosis. In the experiments reported here, the dietary lithocholic acid may represent this irritating material, or result in production of the irritant. The synergistic effect of cholic acid or cholesterol, neither of which produces the ductular cell reaction when fed alone or in combination, is not understood. It is also interesting that feeding the combination of lithocholic acid, cholic acid and cholesterol results in only a minimal reaction. There is some experimental evidence to suggest that the irritant may be the product of an antigen antibody complex in the periductular tissues(8,12). By immunohistochemical technics a non-glycogenic, periodic acid Schiff (PAS) positive antigen (mucopolysaccharide) has been shown to be present in ductule cells and bile in several types of liver diseases(8,12). This material may represent the irritant, or the antigen may elicit antibody formation and the irritant material may be the product of an immunologic reaction. It is possible that toxic chemicals (butter yellow, ethionine, etc.) damage hepatocytes or ductule cells with the production of the PAS positive antigen. However, in the chicken livers with the ductular cell reaction, abnormal deposits of non-glycogenic PAS positive material were not observed.

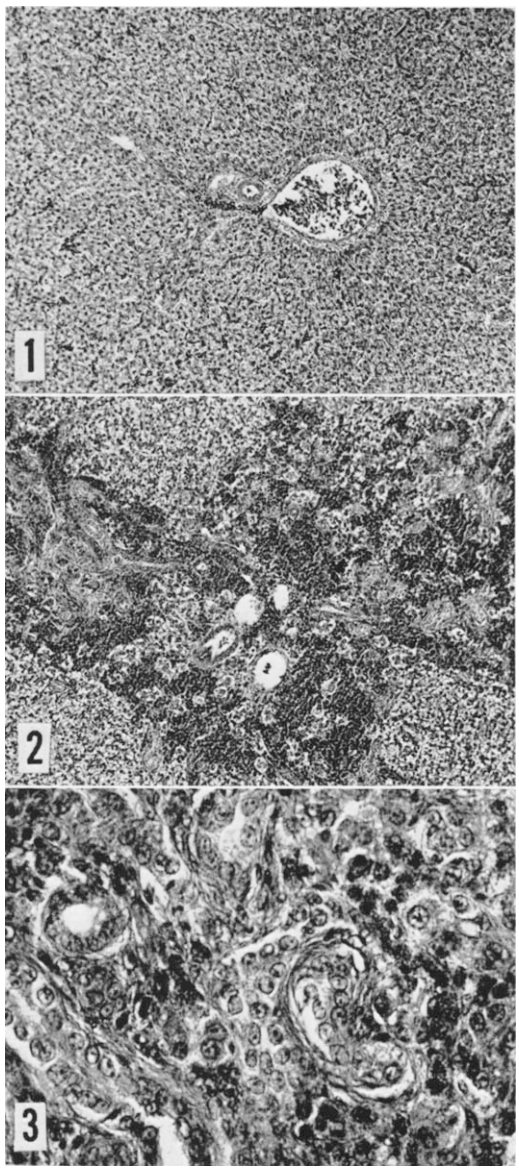

FIG. 1. A normal hepatic triad in liver of a chicken fed the basal diet without additives. Hematoxylin and eosin (H&E) stain. × 75.

FIG. 2. Proliferation of bile ductules in liver of a chicken fed the basal diet to which lithocholic acid (0.2%) was added. The dark staining cells are polymorphonuclear leukocytes. H&E stain. × 75.

FIG. 3. Proliferated ducts, ductules and ductular cells (oval cells) in liver of a chicken fed the basal diet to which lithocholic acid (0.2%) and cholic acid (0.2%) were added. H&E stain. × 375.

Whatever the mechanism involved in the study reported here, it is dependent on the dietary presence of lithocholic acid since removal of this bile acid from the diet results in regression of the proliferated bile ductules, the accompanying fibrosis and the inflammatory cells.

Summary. Dietary lithocholic acid alone and in combination with cholic acid or cholesterol has been shown to induce the ductular cell reaction in the livers of chickens.

Dietary cholic acid, deoxycholic acid, or cholesterol did not alter liver structure. Removal of lithocholic acid from the diet resulted in regression of the reaction.

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Some Effects of Endotoxin upon Plasma Iron Turnover in the Rat. (28301)

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Previous investigations in our laboratory indicated that injection of very small amounts of endotoxin would cause a pronounced decrease in the plasma iron concentration of normal rats(1,2). These lipopolysaccharides from bacteria may be responsible for the decreased plasma iron concentration frequently shown during infection(3,4). The plasma iron in patients or animals with infection disappears rapidly(5,6), and has been shown to be diverted to the liver and spleen instead of the bone marrow(7).

Adrenalectomy or hypophysectomy caused a decrease in plasma iron, but these effects were not directly related to the hypoferremia of infection(8,9). Freireich *et al.*(10) have suggested that the decreased plasma iron during inflammation was due to a decreased return of iron from recently destroyed erythrocytes to the plasma. Other investigators have suggested decreased synthesis(11) or increased catabolism(12) of the iron-binding protein of the plasma. Considerable evidence has been obtained to indicate that the reticuloendothelial system (RES) may be involved in the decrease of plasma iron during infection(10,13,14).

Endotoxins should be useful in studies on the mechanisms of plasma iron lowering, since a rapid decrease and a return to normal can be obtained within a very few hours(2). In the present investigation plasma iron turnover was studied during the period in which plasma iron was decreasing rapidly after an injection of endotoxin.

Methods. Female Holtzman rats weighing 200 to 250 g were used. Each experimental animal received 100 μg of lipopolysaccharide from *Escherichia coli* 055.B5 obtained from Difco Laboratories, Detroit, Mich. Plasma iron concentration was measured by the 2, 2′, 2″-terpyridine method described by Schade *et al.*(15).

Plasma pooled from several rats was incubated with Fe^{59} (specific activity 1-2 μc/μg). One-half milliliter of plasma containing 0.5 to 2 μc of radioiron was injected intravenously into each rat. Blood samples (0.5 ml) were obtained by cardiac puncture at 30, 60, 90, and 120 minutes after injecting radioiron. The effect of the removal of this volume of blood was checked by sampling groups of 3 normal rats at only one of these 4 intervals. The activities obtained in this manner cor-