Action of Lithocholic Acid in the Germ-Free Chick.*† (28345)

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Feeding of lithocholic acid to chicks and hens caused a rise in their serum cholesterol in work of Edwards(1) and Edwards *et al.* (2). When this compound was fed to mice or rats it caused a depression in serum cholesterol in the work of Beher *et al.*(3), Howe *et al.*(4) and Portman and Bruno (5). Recently Howe and Bosshardt(6) demonstrated that in the presence of a mixture of succinylsulfathiazole and oxytetracycline in the diet of mice, lithocholic acid no longer had a serum cholesterol-depressing effect while hyodeoxycholic acid was still effective. They interpreted these findings to mean that lithocholic acid was active by virtue of conversion to an unidentified compound under the influence of the intestinal bacteria. The investigation reported here was carried out to determine if the cholesterol elevating action of lithocholic acid in the chick might also be due to some other compound produced from lithocholic acid in the chicken's gastrointestinal tract.

Experimental. Four plastic canopy germ-free isolators (American Sterilizer Co.) were used for the experiment. The isolators were sterilized by spraying with 30% beta-propiolactone solution; each air filter (Fiber-glass 50) was sterilized with 1 ml methyl bromide; water bottles, cages and other equipment were autoclaved; feed was sterilized with ethylene oxide.

Eggs of Single Comb White Leghorns were obtained from a local hatchery after 18 days of incubation. The eggs were washed in detergent and passed through 2% $HgCl_2$ solution into the isolators. Twelve eggs were placed in each isolator. After hatching, 6 chicks were placed in each of 2 wire cages in each isolator. The basal ration was fed to the chicks in one cage and the lithocholic ration to the chicks in the other cage.

Cultures were taken daily for 3 days and each 3 days thereafter. Swabs were made of surfaces and droppings, passed to the outside and cultured on trypticase-soy agar and thioglycollate broth at 37°C and Sabouraud-maltose agar at 25°C.

Two days after hatching, two units were contaminated by addition of fresh chicken droppings to the drinking water.

The diet used in this study was composed primarily of corn and soybean oil meal with supplemental vitamins and minerals to meet the chick's requirement. It did not contain any animal protein which might contribute bile acids, nor did it contain an antibiotic. The lithocholic acid used in the experiment was obtained from Nutritional Biochemicals Corp. When added to the diet, 0.2% lithocholic acid was substituted for 0.2% corn.

After the chicks received the experimental diets for 2 weeks, the germ-free units were opened. The chicks were immediately weighed and heparinized blood samples to be used for plasma cholesterol determination by the method of Schoenheimer and Sperry(7) were taken by heart puncture. The chicks were sacrificed and the livers removed and weighed. Lipid was extracted from the livers using the method of Folch *et al.*(8).

The amount of lipid material extracted was measured by weighing an aliquot. Aliquots were also taken for cholesterol determination and for fatty acid analysis with gas-liquid chromatography. Careful post-mortem examination was made for any gross changes or abnormalities. The results were analyzed for statistical differences by the Multiple Range Test(9).

Results and discussion. The 2 units that were not purposely contaminated remained germ-free throughout the trial. The growth rate of the chicks fed lithocholic acid was

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TABLE I. Effect of Germ-Free Environment on Action of Lithocholic Acid in Chicks.

	Conventional		Germ-free	
	Control	Lithocholic	Control	Lithocholic
2 wk wt—g	124	83	113	80
Plasma chol—mg/100 ml	179	529	218	620
Liver wt of body wt—%	3.7	9.6	3.4	11.0
" lipid—% fresh wt	5.6	4.9	6.2	4.8
" " —mg	264	457	238	458
" chol—mg	12.5	27.9	9.9	31.4
" lipid fatty acid composition—expressed as % of total fatty acids				
14:0*	.2	.1	.1	.2
14:1	.0	.0	.0	.1
16:0	18.5	31.4	20.3	17.8
16:1	1.3	1.5	1.1	.4
18:0	24.9	22.5	21.9	22.7
18:1	21.3	26.8	22.9	23.7
18:2	18.7	12.2	19.8	22.2
18:3	.0	.0	.0	.4
18:4	.0	.0	.0	.1
20:3	.3	.2	.7	1.1
20:4	14.7	5.3	12.7	11.4

* Carbon chain length: No. of double bonds—tentative identification is based on retention time and hydrogenation to an acid with a retention time similar to the saturated acid of that carbon series.

significantly slower than that of the controls in both conventional and germ-free environments (Table I). The feeding of lithocholic acid increased plasma cholesterol and size of the liver. It decreased the amount of liver lipid expressed as a percent of the liver, but increased the total amount of liver lipids. No differences between conventional and germ-free environments were evident from the afore-mentioned measurements. The most striking gross observation was the almost complete lack of gall bladders in the birds fed lithocholic acid in both environments.

The data on fatty acid composition of liver lipids were much more variable than those previously reported from this laboratory(10) with the result that no treatment differences were noted by statistical analysis. However, the chicks that were fed lithocholic acid in the conventional environment had lower levels of linoleic and arachidonic acid in their livers than the birds under the other treatments.

The results indicate that the serum cholesterol elevating effect of lithocholic acid in chicks is due to lithocholic acid and not some other compound that might be produced from lithocholic acid by microbial action in the gastrointestinal tract of the chick. While the actual amount of bile produced by a chicken fed lithocholic acid was not measured, all the evidence obtained in this study indicates that lithocholic acid greatly decreased bile production and also decreased the metabolic conversion of cholesterol to bile salts. Thus, the high level of plasma and liver cholesterol as well as the extremely high level of total liver lipids would indicate that lithocholic acid disrupts lipid metabolism in this organ.

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