prenatal period is relatively greater than that during any one of the 3 postnatal periods which we studied. This difference raises the question of whether some maternal and/or placental factor(s) other than corticoids contribute(s) to the compensatory hypertrophy which occurred before birth.

Summary. In our experiments, both ponderal and histologic observations on the adrenal support the view that in the rat the hypophysis-adrenal axis begins to function before birth. After birth, there is an hiatus of 3 days during which the function is significantly reduced.

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Dietary Bile Acids and Lipid Metabolism. VI. Protective Effect of Cholic Acid in Lithocholic Acid Fed Chicks. (29169)

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Dietary lithocholic acid has been shown to depress body weight gain and increase plasma lipids and liver size of chicks(1-4). The increased liver size of lithocholic acid-fed chicks has been shown to be the result of a bile ductule proliferation(5).

The deleterious effects of lithocholic acid can be prevented, at least for a 3- or 4-week period, by simultaneously feeding cholic acid (4). Data presented here demonstrate that cholic acid protects chicks against the effects of dietary lithocholic acid for periods of up to 12 weeks.

Experimental. Male Hy-line White Leghorn chicks were fed a stock diet for 2 weeks. The chicks were then assigned to the experimental groups in such a way as to have almost identical average initial weights for all groups (128-130 g).

The chicks were reared in heated cages having raised wire floors. Food and water were supplied *ad libitum.* Body weight and food consumption were determined at weekly intervals. The basal diet employed was identical to that described previously(4). Lithocholic and cholic acids when fed were added at a level of 0.2% of the diet at the expense of glucose. Twenty-eight chicks were started in each of the 6 experimental groups as shown in Table I. Seven chicks from each group were sacrificed after having received the diets for 3, 6, 9 and 12 weeks.

At time of sacrifice, blood was obtained by cardiac puncture using a heparinized syringe. The liver was excised, blotted to remove excess blood and weighed; a slice of liver was fixed in neutral buffered formalin and the remainder was frozen and stored at —20°C for chemical analysis. Plasma and liver lipids

TABLE I. Experimental Design.

Exp group	Dietary variables
1	Control
2	.2% Lithocholic acid
3	*Idem* + .2% cholic acid
4	2% Cholesterol
5	.2% Lithocholic acid + 2% cholesterol
6	*Idem* + .2% cholic acid

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TABLE II. Body Weight, Liver Size, Plasma and Liver Lipids of Chicks Fed Lithocholic or Lithocholic and Cholic Acid-Supplemented Diets Containing or Free of Cholesterol for 3, 6, 9 or 12 Weeks.

			Plasma		Liver†			
Treatment group*		Body wt, g	Cholesterol, mg/100 ml	Lipid phosphorus, mg/100 ml	Size, % body wt	Fat, %	Cholesterol, mg/g	Lipid phosphorus, mg/g
3 wk	1	394 ± 35‡	127 ± 31	11.7 ± 2.6	2.2 ± .3	4.0 ± .4	3.1 ± .2	.91 ± .11
	2	314 ± 55	421 ± 131	32.9 ± 6.4	6.9 ± 2.4	4.7 ± 1.1	4.2 ± .5	1.04 ± .09
	3	380 ± 21	244 ± 50	24.5 ± 3.5	4.6 ± .7	4.4 ± .5	3.5 ± .6	.98 ± .10
	4	426 ± 38	296 ± 79	12.9 ± 1.2	3.0 ± .3	5.8 ± .9	15.7 ± 3.7	.87 ± .13
	5	360 ± 25	502 ± 90	26.7 ± 3.6	5.1 ± .7	4.4 ± .4	7.9 ± 1.3	.90 ± .06
	6	369 ± 19	379 ± 140	14.4 ± 2.8	3.5 ± .4	5.3 ± .6	12.9 ± 4.3	.98 ± .08
6 wk	1	687 ± 47	109 ± 12	9.3 ± 1.1	2.2 ± .2	4.4 ± .5	3.7 ± .3	.95 ± .08
	2	601 ± 95	341 ± 87	23.0 ± 7.7	6.8 ± 2.1	4.1 ± .4	4.6 ± .5	.93 ± .14
	3	688 ± 81	271 ± 44	19.7 ± 1.0	4.4 ± .8	4.0 ± .3	4.4 ± .3	.95 ± .12
	4	747 ± 39	460 ± 140	15.8 ± 2.8	2.7 ± .2	6.6 ± .8	19.2 ± 4.1	1.09 ± .13
	5	650 ± 93	658 ± 211	28.3 ± 7.2	6.2 ± 2.5	5.1 ± .7	9.1 ± 2.8	.99 ± .10
	6	670 ± 71	333 ± 122	14.6 ± 1.8	2.6 ± .3	6.5 ± 1.2	15.1 ± 5.9	1.03 ± .10
9 wk	1	1052 ± 65	118 ± 24	6.9 ± .8	1.8 ± .1	4.2 ± .3	3.6 ± .3	1.07 ± .03
	2	828 ± 142	556 ± 172	27.7 ± 7.0	9.0 ± 2.4	3.9 ± .6	4.8 ± .8	.98 ± .09
	3	997 ± 59	316 ± 47	16.6 ± 4.5	4.6 ± 1.2	3.9 ± .2	4.3 ± .2	.98 ± .08
	4	993 ± 76	453 ± 156	9.0 ± 2.4	2.3 ± .3	5.5 ± .6	13.5 ± 1.9	.85 ± .09
	5	934 ± 77	785 ± 315	19.9 ± 9.5	6.3 ± 2.5	4.1 ± .6	8.0 ± 1.5	.90 ± .11
	6	992 ± 68	437 ± 172	9.2 ± 1.9	2.5 ± .3	6.7 ± .5	16.6 ± 1.9	1.08 ± .23
12 wk	1	1439 ± 106	123 ± 16	11.2 ± 1.5	2.0 ± .1	5.2 ± .5	4.1 ± .3	1.05 ± .07
	2	1015 ± 201	467 ± 62	23.2 ± 5.4	8.7 ± 2.7	4.0 ± .3	5.2 ± .4	.90 ± .07
	3	1318 ± 166	299 ± 39	18.9 ± 3.6	5.8 ± 1.5	4.3 ± .6	5.3 ± .3	.91 ± .15
	4	1414 ± 138	356 ± 131	11.6 ± 2.8	2.4 ± .2	7.1 ± 1.4	21.7 ± 6.4	1.08 ± .11
	5	1177 ± 168	944 ± 395	24.9 ± 8.0	6.8 ± 2.7	4.7 ± 1.0	8.2 ± 1.8	.87 ± .10
	6	1390 ± 89	464 ± 150	16.2 ± 4.4	2.3 ± .2	7.3 ± .9	20.6 ± 4.8	1.05 ± .08

* Groups 1-3 received a cholesterol-free diet; Groups 4-6 were fed the same diet supplemented with 2% cholesterol; in addition, Groups 2 and 5 received a supplement of .2% lithocholic acid; Groups 3 and 6 received supplements of .2% each of lithocholic and cholic acids.

† All liver values expressed on a wet weight basis.

‡ Mean for 7 chicks ± standard deviation.

were determined as previously described(6). The formalin-fixed tissue was prepared for microscopic examination as previously described(5). Results were statistically evaluated by means of the "t" test.

Results. Results are presented in Table II. Body weight was significantly lower in chicks fed lithocholic acid. However, the simultaneous addition of cholic acid overcame the body weight-depressing effect of lithocholic acid. Dietary cholesterol did not influence body weight. Plasma cholesterol and lipid phosphorus levels were significantly increased by lithocholic acid feeding. The lipemia induced by lithocholic acid feeding was less marked when cholic acid was fed simultaneously to chicks receiving a cholesterol-free diet and was absent in cholesterol-fed chicks receiving a dietary supplement of both bile acids. Dietary cholesterol, as would have been expected, increased plasma cholesterol levels significantly. Chicks fed a cholesterol-free diet had a significantly greater liver size when fed lithocholic acid; cholic acid partially overcame this increase. As shown in Table II, cholic acid completely prevented the increase in liver size resulting from lithocholic acid supplementation of a cholesterol-containing diet.

Liver fat was decreased slightly by lithocholic acid in chicks fed a cholesterol-free diet; however, this difference was significant only at the 12th week. Dietary cholesterol significantly increased liver fat and lithocholic acid decreased liver fat significantly in the cholesterol-fed chick. Addition of cholic acid to the lithocholic acid-cholesterol-supplemented diet resulted in liver lipid values intermediate to and significantly different from those observed in chicks fed cholesterol or cholesterol and lithocholic acid. Liver cholesterol levels were significantly increased in chicks fed lithocholic acid. Addition of cholic acid to the lithocholic acid-supplemented diet resulted in slightly lower values than those observed in chicks fed lithocholic acid alone, but significantly higher than those from chicks fed the unsupplemented control diet. In the cholesterol-fed chick, lithocholic acid decreased liver cholesterol levels, while chicks fed both cholic and lithocholic acids had liver cholesterol levels which were not significantly different from those of chicks fed the control cholesterol-supplemented diet. Liver lipid phosphorus levels were not significantly altered by the dietary treatments used.

Microscopic examination of the livers revealed a marked ductular proliferation, as previously described, resulting from lithocholic acid ingestion(5). The degree of proliferation induced by lithocholic acid ingestion did not appear to be influenced by cholic acid in chicks fed a cholesterol-free diet. In the cholesterol-fed chick, however, cholic acid markedly influenced the degree of ductular proliferation. All chicks fed lithocholic acid in combination with cholesterol showed a very marked proliferation of bile ductules. Only a very modest involvement was seen in the livers of chicks fed diets containing the combined supplement of cholesterol, cholic and lithocholic acids.

Discussion. The data presented demonstrate that cholic acid somehow protects chicks from the deleterious effects of dietary lithocholic acid. In chicks fed cholesterol, cholic acid affords virtually complete protection against both the biochemical and morphological changes induced by dietary lithocholic acid. However, in chicks fed a cholesterol-free diet, only partial protection is afforded against the biochemical changes noted and no apparent protection is given against the morphological changes seen in the liver as a result of lithocholic acid feeding.

Bile ductule proliferation is induced by a number of different agents both carcinogenic and noncarcinogenic(7) and by biliary obstruction or ligation(8). Recent studies have added lithocholic acid to the list of compounds capable of inducing hyperplasia of bile ductules(2,3,4,5). As pointed out by Songster *et al*(9), the morphological appearance of the biliary proliferation seen in these various intoxications is sufficiently similar to suggest a common mechanism. Songster *et al* (9) suggest the intriguing hypothesis that bile duct hyperplasia may result from an alteration of bile acid metabolism. The present study demonstrating that biliary hyperplasia or the "ductular cell reaction"(7) can be induced by ingestion of a bile acid and that ingestion of a second bile acid may prevent or at least reduce the severity of the hyperplasia, would strengthen such a hypothesis. The possibility remains that cholic acid may act, not on a metabolic pathway, but simply by interfering with the absorption of lithocholic acid.

Summary. Dietary lithocholic acid increased plasma lipid levels, decreased liver fat, increased liver size and induced a marked biliary hyperplasia in chicks fed either a cholesterol-free or supplmented diet. Lithocholic acid increased liver cholesterol levels in chicks fed a cholesterol-free diet and decreased liver cholesterol in chicks fed a cholesterol-supplemented diet. Cholic acid supplementation partially prevented the changes in liver size and plasma liver lipid levels resulting from ingestion of a lithocholic acid-supplemented cholesterol-free diet. In cholesterol-fed chicks, cholic acid afforded almost complete protection against the effects of dietary lithocholic acid.

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