Dietary Bile Acids and Lipid Metabolism. I. Influence on Lipids and Liver Size of Chicks. (28668)

G. A. LEVEILLE, H. E. SAUBERLICH AND R. D. HUNT
U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colo.

Several workers have demonstrated an influence of dietary bile acids on plasma and hepatic cholesterol levels of mice and rats (2,6). The results of these studies have shown dietary cholic acid to be hypercholesterolemic. Lithocholic acid, on the other hand, lowered blood and liver cholesterol levels of mice and rats.

Little work has been reported on the influence of dietary bile acids in the chick. Hegsted *et al*(7) demonstrated a cholesterol elevating effect of cholic acid in cholesterol-fed chicks. More recently, Edwards(8) reported that cholic and hyodeoxycholic acids did not influence serum cholesterol levels of chicks fed a cholesterol-free diet, but that lithocholic acid, in contrast to its effect in mice and rats, was hypercholesterolemic. These results suggest that chicks fed a cholesterol-free or supplemented diet respond differently to cholic acid supplementation.

The studies herein reported were undertaken to determine the influence of dietary cholic and lithocholic acids on lipid metabolism in growing chicks fed a cholesterol-free or supplemented diet.

Experimental. Male Hy-line chicks were used in all experiments. Chicks were distributed to the various experimental groups on the basis of body weight in such a manner as to have almost identical mean weights for all groups in each study. The length of the experimental periods is indicated in the results section.

The composition of the diet employed is shown in Table I. Bile acids and cholesterol were added to the diet at a level of 0.2 and 2.0 g/100 g of diet, respectively. All additions to the basal diet were made at the expense of glucose.

The chicks were housed in heated cages having raised wire floors. Food and water were supplied *ad libitum*. Body weight and food consumption were determined at weekly intervals.

Blood was drawn by cardiac puncture with a heparinized syringe at weekly intervals in Exp. 3 and at termination of each experiment. At termination of Exp. 1 and 2, the animals were sacrificed and livers were excised, freed of excess blood and weighed. Livers were then frozen and stored at —20°C until analyzed.

Plasma and liver lipids were determined as previously described(10). The data were analyzed statistically by "t" test.

Results. The results are presented in Tables II, III and IV for Exp. 1, 2 and 3, respectively.

Experiment 1 (Table II). The chicks used in this experiment were prefed a stock diet for one week and the experimental diets for 3 weeks.

Chicks fed lithocholic acid and cholesterol gained less weight than did chicks fed the basal diet ($P < 0.001$). Dietary lithocholic acid also increased plasma cholesterol and lipid phosphorus ($P < 0.001$) and increased liver size ($P < 0.001$), both in terms of weight and as a percentage of body weight. Lithocholic acid also decreased liver fat of chicks fed the cholesterol-supplemented diet ($P < 0.005$). Liver cholesterol levels of chicks fed a cholesterol-free diet were increased ($P < 0.01$), while those of chicks

TABLE I. Composition of Basal Diet.

Ingredients	Amount, g/100 g diet
Assay protein C-1*	29.40
Glycine	.40†
DL-methionine	.30†
Vitamin mixture‡	.40
Salt mixture‡	5.31
Choline Cl	.20
Non-nutritive fiber	4.00
Corn oil	5.00
Glucose	to 100

* Archer-Daniels Corp., Midland, Mich.
† In Exp 1 all diets contained 0.20% glycine and 0.35% DL-methionine.
‡ For composition see Leveille *et al.*(9).

TABLE II. Body Weight Gain, Liver Weight and Plasma and Liver Lipids of Chicks Fed Bile Acids With and Without Cholesterol.

Experiment 1*

		Plasma		Liver§			
Bile acid†	Body wt gain, g‡	Cholesterol, mg/100 ml	Lipid P, mg/100 ml	Weight, % body wt	Fat, %	Cholesterol, mg/g	Lipid P, mg/g
			No added cholesterol				
None	191 ± 24‖	140 ± 12	13.51 ± 1.55	2.8 ± .6	5.3 ± .3	4.4 ± .2	1.32 ± .25
Cholic	189 ± 15	157 ± 21	13.10 ± 1.22	2.5 ± .3	5.4 ± .5	4.0 ± .3	1.18 ± .08
Lithocholic	184 ± 22	459 ±117	32.12 ± 6.99	5.7 ± .3	5.1 ± .5	5.0 ± .4	1.11 ± .08
Cholic + lithocholic	182 ± 28	228 ± 93	16.86 ± 2.59	3.4 ± .4	3.4 ± .8	3.8 ± .7	.72 ± .14
			2% added cholesterol				
None	221 ± 18	344 ± 84	10.12 ± 1.44	2.9 ± .2	8.2 ± 1.6	29.2 ± 9.7	.97 ± .06
Cholic	199 ± 15	697 ±180	13.02 ± 2.40	3.0 ± .2	8.0 ± 1.2	28.3 ± 7.0	.94 ± .10
Lithocholic	155 ± 36	743 ±196	31.09 ± 9.56	6.5 ±2.1	4.8 ± .4	8.1 ± 1.4	.85 ± .06
Cholic + lithocholic	194 ± 17	495 ±219	10.92 ± 1.93	2.6 ± .3	6.2 ± .8	19.6 ± 6.2	.91 ± .10

* The chicks in this study were fed the experimental diets from 7th to 28th day of age.
† All bile acids added at a level of 0.2% of diet.
‡ Weight gain for 21-day experimental period.
§ All liver values expressed on a wet weight basis.
‖ Mean for 8 animals ± standard deviation.

fed cholesterol were decreased ($P < 0.001$) by lithocholic acid. The effects of lithocholic acid were partially or completely reversed by simultaneous feeding of cholic acid. The weight gain of chicks fed lithocholic acid in the cholesterol-supplemented diet was significantly improved when cholic acid was also fed ($P < 0.025$). The elevated plasma cholesterol and lipid phosphorus levels of lithocholic acid-fed chicks were reduced by cholic acid supplementation, particularly in chicks fed cholesterol. The increased liver size of lithocholic acid-fed chicks was also reduced by cholic acid supplementation.

Cholic acid did not significantly alter any of the parameters evaluated in chicks fed a cholesterol-free diet. In chicks fed cholesterol, however, cholic acid significantly increased plasma cholesterol ($P < 0.001$) and lipid phosphorus ($P < 0.025$).

No appreciable changes were observed in liver lipid phosphorus values as a result of the treatments employed.

*Experiment 2, (Table III).** Exp. 2 was designed to test the reproducibility of the results obtained with cholic and lithocholic acids in Exp. 1. Four-week old chicks which had been fed a stock diet were used and were fed the experimental diets for 4 weeks.

* Some of the data from Experiment 2 have been reported in a preliminary note, Leveille *et al.*(1).

The effects of lithocholic acid supplementation are in good agreement with those obtained in Exp. 1. Lithocholic acid depressed weight gain of cholesterol-fed chicks ($P < 0.01$), increased plasma cholesterol and lipid phosphorus levels ($P < 0.001$), increased liver size ($P < 0.001$) and decreased liver fat ($P < 0.005$). Liver cholesterol was increased in chicks fed the cholesterol-free diet ($P < 0.001$) and decreased in cholesterol-fed chicks ($P < 0.001$).

As in Exp. 1, cholic acid did not significantly alter weight gain, liver size or liver lipids but significantly increased plasma cholesterol and lipid phosphorus of cholesterol-fed chicks ($P < 0.05$). In contrast to the results of Exp. 1, chicks fed cholic acid without a cholesterol supplement had significantly higher plasma cholesterol and lipid phosphorus levels ($P < 0.005$). Liver lipid phosphorus values remained unchanged.

Experiment 3 (Table IV). Exp. 3 was designed to determine whether the increased plasma cholesterol level noted in older chicks fed a cholic acid-supplemented, cholesterol-free diet (Exp. 2) might be related to age. In Exp. 3, chicks were prefed a stock diet for the first week and received the experimental diets for 8 weeks (7th to 63rd day

TABLE III. Body Weight Gain, Liver Weight and Plasma and Liver Lipids of Chicks Fed Bile Acids With and Without Cholesterol.
Experiment 2*

		Plasma		Liver§			
Bile acid†	Body wt gain, g‡	Cholesterol, mg/100 ml	Lipid P, mg/100 ml	Weight, % body wt	Fat, %	Cholesterol, mg/g	Lipid P, mg/g
			No added cholesterol				
None	338 ± 74‖	153 ± 16	13.13 ± 1.53	2.63 ± .22	5.6 ± .6	4.1 ± .3	.85 ± .03
Cholic	294 ± 44	211 ± 29	18.11 ± 1.95	2.87 ± .22	5.8 ± 1.4	4.5 ± .5	.96 ± .10
Lithocholic	291 ± 32	458 ± 99	33.72 ± 6.56	5.87 ± 1.08	4.0 ± .9	5.3 ± .3	.96 ± .08
Cholic + lithocholic	320 ± 29	264 ± 35	20.10 ± 1.89	3.92 ± .38	5.2 ± .9	4.8 ± .8	.97 ± .16
			2% added cholesterol				
None	330 ± 40	450 ± 96	11.06 ± 1.46	2.83 ± .05	6.9 ± 1.2	12.1 ± 1.1	.98 ± .10
Cholic	332 ± 47	622 ± 142	13.60 ± 1.43	2.93 ± .33	6.4 ± 1.1	13.6 ± 2.7	.89 ± .09
Lithocholic	258 ± 35	739 ± 170	29.91 ± 4.29	5.37 ± .59	4.9 ± .4	8.2 ± .9	.87 ± .08
Cholic + lithocholic	261 ± 26	353 ± 81	11.41 ± 1.55	2.95 ± .14	5.7 ± .7	11.1 ± 2.1	.86 ± .03

* The chicks used in this study were fed the experimental diets from 28th to 56th day of age.
† All bile acids added at a level of 0.2% of diet.
‡ Weight gain for 28-day experimental period.
§ Liver values expressed on a wet weight basis.
‖ Mean for 6 animals ± standard deviation.

TABLE IV. Cholesterol and Lipid Phosphorus Levels of Plasma from Chicks Fed Diets With and Without Added Cholic Acid.
Experiment 3

	Cholesterol, mg/100 ml		Lipid phosphorus, mg/100 ml		No. of determinations	
Wk of exp*	Control	Cholic acid fed†	Control	Cholic acid fed†	Control	Cholic acid fed†
1	172 ± 29‡	210 ± 27	12.4 ± 1.7	15.4 ± 2.1	6	6
2	142 ± 18	148 ± 19	15.0 ± 3.5	14.5 ± 3.5	6	6
3	134 ± 22	149 ± 25	10.5 ± 1.2	12.4 ± 2.2	6	4
4	167 ± 21	166 ± 32	11.8 ± 2.2	12.3 ± 2.3	11	9
5	162 ± 22	156 ± 14	9.3 ± 1.0	9.2 ± .7	11	9
6	113 ± 9	118 ± 11	11.3 ± 1.4	11.1 ± 1.0	10	8
7	183 ± 12	186 ± 20	15.0 ± 1.4	14.6 ± 1.7	11	9
8	139 ± 14	147 ± 19	11.2 ± .9	12.3 ± 1.7	11	8

* Chicks were prefed a stock diet for 1 week, therefore value for week 1 represents sample taken on 14th day of age.
† Cholic acid fed at a level of 0.2% of diet.
‡ Mean ± standard deviation for number of determinations shown.

of age). Blood samples were obtained at weekly intervals for cholesterol and lipid phosphorus determinations. Cholic acid supplementation did not significantly alter plasma lipids at any time during the 8-week experimental period (Table IV).

Discussion. The data presented demonstrate that dietary lithocholic acid decreases body weight gain, increases liver size, plasma cholesterol and lipid phosphorus levels and decreases liver fat in the presence or absence of dietary cholesterol. Dietary lithocholic acid increased the liver cholesterol level of chicks fed a cholesterol-free diet and depressed liver cholesterol in cholesterol-fed chicks.

The increased liver size and plasma lipid levels found in lithocholic acid-fed chicks were partially reversed when cholic acid was fed simultaneously in a cholesterol-free diet. In chicks fed a cholesterol-supplemented diet, cholic acid afforded almost complete protection from the effects of lithocholic acid. The protective effect of cholic acid was observed after feeding the experimental diets for 3 or 4 weeks and similar results have been reported concerning plasma cholesterol levels (8).

The mechanism(s) by which dietary lithocholic acid exerts the effects reported is not clear. Dietary lithocholic acid has been reported to induce liver cirrhosis in the rabbit (11) and Iguana iguana(12) and parenterally administered lithocholic acid has been shown to be pyrogenic in man(13). The increase in liver size observed appears to be the result of a biliary hyperplasia(1); however, the etiology of the lesion remains obscure.

The hypercholesterolemic effect of cholic acid when fed in conjunction with cholesterol and its lack of effect in a cholesterol-free diet, observed in these studies, is in accord with the reports of Hegsted *et al*(7) and Edwards (8). The indication from Experiment 2 that cholic acid might be hypercholesterolemic in older chicks fed a cholesterol-free diet was not borne out by the data obtained in an 8-week study (Exp. 3). Dietary cholic acid has been reported to increase liver cholesterol levels in mice(3) and rats(6); however, this bile acid did not significantly alter liver cholesterol levels in these studies with chicks.

Summary. Dietary lithocholic acid elevated plasma cholesterol and lipid phosphorus levels, and increased liver size of growing chicks. In cholesterol-fed chicks, lithocholic acid depressed liver lipid and cholesterol levels. The effects of lithocholic acid were partially reversed by cholic acid in chicks fed a cholesterol-free diet and almost completely reversed in chicks fed cholesterol. The plasma cholesterol level of cholesterol-fed chicks was increased by cholic acid feeding.

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1. Leveille, G. A., Sauberlich, H. E., Hunt, R. D., *Poultry Sci.*, 1962, v41, 1991.
2. Beher, W. T., Anthony, W. L., Baker, G. D., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 317.
3. Beher, W. T., Baker, G. D., Anthony, W. L., *ibid.*, 1960, v103, 385.
4. Hegsted, D. M., Andrus, S. B., Gotsis, A., Portman, O. W., *J. Nutrition*, 1957, v63, 273.
5. Howe, E. E., Bosshardt, D. K., Huff, J. W., *ibid.*, 1960, v72, 379.
6. Portman, O. W., Bruno, D., *ibid.*, 1961, v73, 329.
7. Hegsted, D. M., Gotsis, A., Stare, F. J., *ibid.*, 1960, v70, 119.
8. Edwards, H. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 742.
9. Leveille, G. A., Sauberlich, H. E., *J. Nutrition*, 1962, v76, 321.
10. ———, PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 300.
11. Holsti, P., *Nature*, 1960, v186, 250.
12. Stolk, A., *Experientia*, 1960, v16, 507.
13. Palmer, R. H., Glickman, P. B., Kappas, A., *J. Clin. Invest.*, 1962, v41, 1573.

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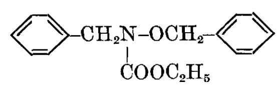
Effect of Benzyl N-Benzyl Carbethoxyhydroxamate (W-398) on Experimental Atherosclerosis and Hypercholesteremia. (28669)

F. M. BERGER, J. F. DOUGLAS, B. J. LUDWIG AND S. MARGOLIN
Wallace Laboratories, Division of Carter Products, Inc., Cranbury, N. J.

The compound benzyl N-benzyl carbethoxyhydroxamate (W-398) was originally described as a chemical intermediate by Jones and Fleck(1), but no reference to its biological effects has been found in the literature. The present paper describes the ability of this compound to reverse experimental atherosclerotic lesions and to reduce the levels of cholesterol and other fatty substances in the blood of certain species of animals. The compound has the following structural formula:



It is a bitter, oily liquid, poorly soluble in water (0.006% at 37°C) but readily miscible with most organic solvents. It is stable in dilute acids or alkali and is not altered by gastric or intestinal juices at 37°C.

Methods. Experimental atherosclerosis was induced in young adult male albino rabbits