Weight Gain and Intestinal Histology of Rats Fed Cholic, Lithocholic, Hyodeoxycholic, Chenodeoxycholic, and Deoxycholic Acids* (33515)

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Studies from this laboratory showed that bile acids inhibit food intake in rats (1) and reduce appetite in man (2), but the mechanism for this effect is unknown. One possibility is that they have a direct effect on the intestinal mucosa with the eventual production of histologic alterations. To test this, we investigated the effect of five bile acids on the reesterification of fatty acids by slices of intestinal mucosa and examined the histology of intestine from rats fed bile acids for 4 weeks.

Methods and Materials. Esterification of oleate-1-^{14}C was determined as described by Dawson and Isselbacher (3). Two feeding experiments were performed:

I. Six groups of 7 male Gofmoor rats weighing 55–70 g were placed in stainless steel wire bottom cages and fed a semisynthetic diet (group I) or a diet fortified with 1.5 g of one of the 5 bile acids for each kg of diet. Body weight of each rat was recorded two to three times weekly and at the end of 26 days, the rats were anesthetized with ether and bled from the abdominal aorta. The intestine, liver, heart, gonads, and thyroid were dissected free, weighed, and sections of heart, liver, stomach with duodenum, and ileum with colon were fixed in both 10% formalin and Zenker's solution. Histological sections from the fixed tissues were prepared and stained with hematoxylin and eosin.

II. Two groups of 6 male rats, with mean weights of 244 and 250 g, were fed a semisynthetic diet, with or without 0.15% deoxycholic acid, for a period of 120 days with frequent measurement of body weight.

Materials. Cholic acid (3α,7α,-12α-trihydroxycholanic acid) was obtained from Eastman Organic Chemicals. Deoxycholic acid (3α,12α-dihydroxycholanic acid) was purchased from Calbiochem. Lithocholic acid (3α-hydroxycholanic acid) and chenodeoxycholic acid (3α, 7α-dihydroxycholanic acid) were purchased from K and K Laboratories and hyodeoxycholic acid (3α,6α-dihydroxycholanic acid) was purchased from Mann Research Laboratories. In the feeding experiments the bile acids were used as supplied, but for the experiments on reesterification they were recrystallized from ethylacetate (chenodeoxycholic acid) or 70% ethanol.

Results. Esterification. Lithocholic and cholic acid were without effect on the incorporation of oleate into lipid by slices of rat intestine at any concentration from 0.01 to 10 m*M*. While chenodeoxycholic acid, hyodeoxycholic acid, and deoxycholic acid all significantly reduced the incorporation of oleic-1-^{14}C into lipid by intestinal slices at 10 m*M* (Fig. 1), hyodeoxycholic acid and deoxycholic acid also reduced esterification at 1 m*M*. At lower concentrations none of the bile acids diminished esterification, or reduced the up-

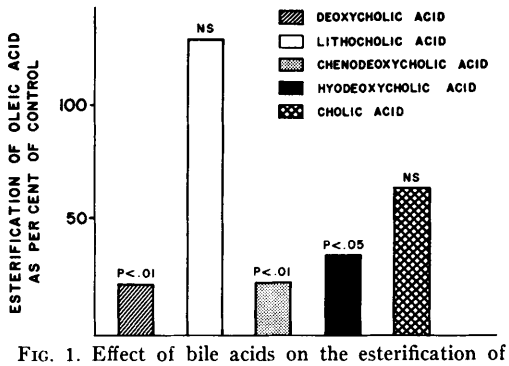

FIG. 1. Effect of bile acids on the esterification of oleate-14C by intestinal slices of the rat. Each bar is the mean of eight replicates incubated with a 10 m*M* concentration of bile acid as described in the text. Three bile acids significantly decreased esterification and two did not.

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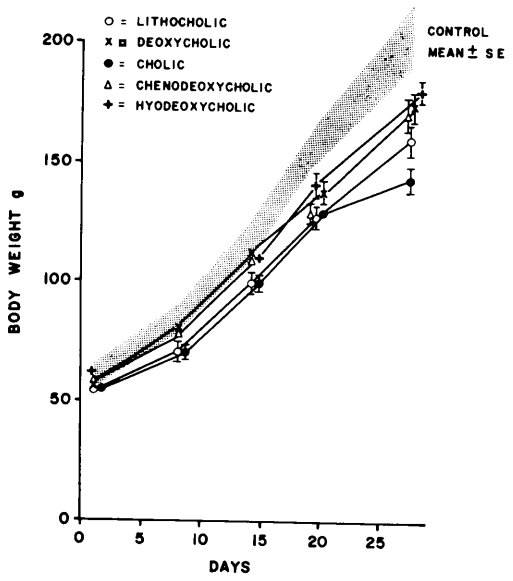

FIG. 2. Effect of bile acids on weight gain of rats. Male albino rats were fed a diet with 0.15% of bile acid. The mean± 2 SE are shown for the control group ($n = 7$) but only the mean for the other 5 groups.

take of radioactive oleic acid by the intestinal slices.

Feeding experiments. The mean body weight of the six groups of rats used in the first feeding experiments were identical at the beginning of the experiment. After the first week, the rats on lithocholic and cholic acids weighed significantly less (Fig. 2). By the end of 3 weeks, the mean weight of all rats receiving bile acids was reduced. The weight of the liver per 100 g of body weight was reduced in rats receiving cholic acid, but the other bile acids had no effect on the weight of the liver (Table I). The weight of the heart per 100 g of body weight was significantly increased in the rats fed cholic and lithocholic acids, but not by the other bile acids. The weight of the gonads and thyroid were unaffected by any of the bile acids. Histologic sections of heart, liver, duodenum, ileum, and colon were examined by Dr. Ronald Hunt, Veterinary pathologist at the New England Regional Primate Center, who was unable to find any evidence of alteration in the histology of these organs as a result of feeding bile acids.

Deoxycholic acid, 0.15%, was fed to six rats for 120 days and the rate of weight gain was slowed for the first 30 days, but there was no detectable effect of the bile acid during the remaining 90 days.

Discussion. Deoxycholic acid is widely used by biochemists to solubilize membranes and it was shown by several groups (3–5) to reduce esterification of fatty acids by intestinal slices *in vitro*. Our data confirm this effect at 1 and 10 m*M* concentrations and show in addition, that at 10 m*M* concentrations, chenodeoxycholic acid and hyodeoxycholic acid also inhibit esterification. Lithocholic and cholic acid had no effect on esterification at any concentration up to 10 m*M*. Since all five bile acids were comparably effective in reducing weight gain, this would suggest that the effect of the dihydroxy bile acids on esterification are not directly related to the mechanism of reduced food intake.

Dawson and Isselbacher (3) showed that when intestinal slices from rats were incu-

TABLE I. Effect of Bile Acids on the Body Weight and the Weight of the Heart and Liver.[a]

Bile acid	No. of animals	Body wt.	Liver (g)	Wt. (g/100 g of body wt.)	Heart (mg)	Wt. (mg/100 g of body wt.)
None	5	205 ± 6[b]	8.3 ± 0.3	4.05 ± 0.15	809 ± 52	390 ± 25
Lithocholic	7	161 ± 6	6.3 ± 0.3	3.91 ± 0.16	740 ± 21	460 ± 13
Deoxycholic	5	165 ± 7	6.9 ± 0.3	4.18 ± 0.24	700 ± 10	420 ± 10
Cholic	7	144 ± 5	5.1 ± 0.2	3.52 ± 0.19	653 ± 18	450 ± 12
Chenodeoxycholic	7	172 ± 7	6.5 ± 0.4	3.75 ± 0.25	717 ± 24	420 ± 14
Hyodeoxycholic	7	181 ± 5	7.2 ± 0.4	3.98 ± 0.20	818 ± 74	450 ± 15

[a] Body and organ weights were measured after 26 days on the bile acids.
[b] Mean ± SEM.

bated with 1 to 5 m*M* deoxycholate, histological damage ensued. Fry and Staffeldt (6) observed histological changes within 8 days in the mucosa of mice fed 2% deoxycholate, but these changes had almost completely resolved after 38 days. Ductular cell hyperplasia of the liver can be readily produced by feeding lithocholic acid to chickens or rabbits (7, 8) and production of similar lesions was observed in rats fed high concentrations of lithocholic acid (9, 10). In our experiments none of the bile acids altered hepatic architecture or produced detectable histological changes in the intestine. This confirms the recent findings of Dawson (11) who failed to produce histological changes in the gut of rats fed deoxycholate *in vivo*. Yet our diet, containing 0.15% of bile acid significantly reduced the rate of weight gain implying that the effect on weight gain occurs without detectable histological alterations.

Retarded weight gain has been reported previously (12, 13) and Visek *et al.* (13) observed that each 0.1% increment in the concentration of deoxycholic acid in the diet produced a 10% reduction in weight gain. We have demonstrated that the parenteral injection of chenodeoxycholic acid and deoxycholic acid acutely reduce food intake in the rat (1) again making it unlikely that a toxic effect on the intestinal mucosa was involved.

Summary. Deoxycholic, hyodeoxycholic, and chenodeoxycholic acid at 1 or 10 m*M* inhibited esterification of oleate *in vitro,* by slices of intestinal mucosa, but lithocholic and cholic acid did not. All five bile acids at a concentration of 0.15% in the diet reduced weight gain over a 30-day period, but none of them produced any distologic alteration in the intestinal mucosa.

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1. Bray, G. A. and Gallagher, T. F., Jr., Federation Proc. **27**, 274 (1968).

2. Bray, G. A. and Gallagher, T. F., Jr., Lancet **1**, 1066 (1968).

3. Dawson, A. M. and Isselbacher, K. J., J. Clin. Invest. **39**, 730 (1960).

4. Donaldson, R. M., Jr., J. Clin. Invest. **44**, 1815 (1965).

5. Dietschy, J. M., Federation Proc. **26**, 1589 (1967).

6. Fry, R. J. M. and Staffeldt, E., Nature **203**, 1396 (1965).

7. Hunt, R. D., Leveille, G. A., and Sauerblich, H. E., Proc. Soc. Exptl. Biol. Med. **113**, 139 (1963).

8. Hunt, R. D., Leveille, G. A., and Sauerblich, H. E., Proc. Soc. Exptl. Biol. Med. **115**, 277 (1964).

9. Palmer, R. H. and Hruban, Z., J. Clin. Invest. **45**, 1255 (1966).

10. Zaki, F. G., Carey, J. B., Jr., Hoffbauer, F. W., and Nivokolo, C., J. Lab. Clin. Med. **69**, 737 (1967).

11. Dawson, A. M., Gut 8, 1 (1967).

12. Howe, E. E., Bosshardt, D. K., and Huff, J. W., J. Nutr. **72**, 379 (1960).

13. Visek, W. J., Dang, H. C., Kirby, S. R., and Sperling, G. A., Proc. Soc. Exptl. Biol. Med. **120**, 48 (1965).

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