Effect of Dietary Bile Acids on *in vivo* Cholesterol Metabolism in the Rat.* (24220)

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Dietary cholesterol by itself does not cause any large amount of cholesterol deposition in rat liver, however, when fed with various bile acids, it produces cholesterol type fatty livers. Previously an increase in intestinal cholesterol absorption was accepted as the most obvious explanation of this phenomenon, but recent balance studies by Pihl(1) have given evidence that this explanation is untenable. Pihl suggested that a retarded rate of cholesterol catabolism coupled with normal rate of absorption would account for cholesterol accumulation in the presence of dietary bile acids. Since Friedman and Byers(2) have shown that accumulated cholesterol must be degraded as it is mobilized, Pihl's theory would seem acceptable. Further evidence is provided by other experiments(3,4) which have shown that bile acids prevent mobilization of accumulated liver cholesterol. The effect of cholic acid on metabolism of cholesterol has been studied by Byers *et al.*(5), who reported that cholic acid treated rats metabolize cholesterol at essentially the same rate as controls. However, their experiment was carried out on bile duct ligated animals which are not physiologically normal. In an effort to explain the hypercholesterolemic effect of bile acids, we have investigated the effects of cholic and dehydrocholic acids on synthesis and mobilization of cholesterol under normal physiological conditions.

Methods. In Exp. A, we studied the effect of cholic acid on cholesterol metabolism; while in a parallel Exp. B, we studied the effect of dehydrocholic acid. Female Sprague-Dawley albino rats—24, weighing 230-260 g in Exp. A, and 24, weighing 180-200 g in Exp. B, were divided into separate control and experimental groups for each of the 2 studies. Both control groups received a basal diet consisting of ground Rockland rat ration with 3% corn oil added; the experimental groups received the same diet supplemented with either 0.5% cholic acid (Exp. A), or 0.5% dehydrocholic acid (Exp. B). All animals were fed *ad lib.* for 3 weeks, during which average food consumption in each group was 18 g/day. Each rat then received an intraperitoneal injection of 50 μc of sodium acetate-1-C^{14} in saline. Six, 24, 48 and 120 hours later, 3 control and 3 experimental rats for each of the 2 experiments were bled by heart puncture and decapitated. Samples of blood, liver, and adrenals were removed for analyses. Total serum cholesterol was determined by the method of Sperry and Webb (6). Serum bile acid was determined by absorption photometry by a combination of the methods of Minibeck and Wilken(7,8). Livers and adrenals were homogenized in a Lourdes multimixer, and total cholesterol determined on aliquots of homogenates by the method of Sperry and Webb. For determinations of radioactivity, blood, liver, and adrenal cholesterol was isolated as the digitonide and counted in a Tracerlab SC-16-Windowless Flow Counter. All samples were of such weight that no self-absorption corrections were necessary. After counting, the weight of cholesterol on the planchets was determined by the Lieberman-Burchard reaction. All radioactivity determinations are reported as specific activity of cholesterol (counts/min/mg cholesterol). To check the rate of acetate oxidation and size of the acetate pool, rate of incorporation of C^{14} into expired CO_2 was determined for both experiments. In each experiment, 8 additional animals were fed the prescribed diets for 3 weeks. Then they received intraperitoneal injections of 50 μc of sodium acetate-1-C^{14} in saline. The expired CO_2 was collected for 6 hours following injection. The collected CO_2 was precipitated as $BaCO_3$ and its activity determined in a windowless flow counter.

Results. Exp. A. As shown in Table I, 3 weeks of cholic acid feeding did not alter se-

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TABLE I. Effect of Dietary Cholic and Dehydrocholic Acids on Tissue Cholesterol Levels and on Serum Bile Acid Levels.

Exp.	Group	Total cholesterol			Bile acid
		Serum, mg %	Liver, mg/g	Adrenal, mg/g	Serum, mg %
A	Control	81.6 ± 13.2	2.60 ± .19	44.7 ± 6.81	2.42 ± .61
	Cholic acid treated (.5%)	84.0 ± 16.9	2.83 ± .22	44.0 ± 5.95	4.25 ±1.03
B	Control	83.0 ± 10.2	2.97 ± .45	37.6 ± 6.27	2.42 ± .61
	Dehydrocholic acid (.5%)	84.6 ± 13.6	3.00 ± .11	42.7 ± 9.55	3.24 ± .89

Statistical comparison of values for control and treated animals:

Cholesterol: Exp. A: Serum, $P = .84$; Liver, $P = .015$; Adrenal, $P = .86$. Exp. B: Serum, $P = .75$; Liver, $P = .88$; Adrenal, $P = .15$.

Bile acid: Exp. A: Serum, $P < .01$. Exp. B: Serum, $P = .14$.

rum or adrenal total cholesterol levels, however, there was a slight but significant increase in liver total cholesterol. Cholic acid treatment resulted in a significant increase in serum bile acid level.

Table II shows that cholic acid greatly inhibited the rate of C^{14} incorporation in serum, liver, and adrenal cholesterol. Furthermore, cholic acid greatly retarded rate of mobilization of cholesterol in these same tissues.

Exp. B. Dietary dehydrocholic acid did not increase serum, liver or adrenal cholesterol levels (Table I). In contrast to cholic acid, dehydrocholic acid produced no significant increase in serum bile acids. However, it must be kept in mind that dehydrocholic acid does not yield a product measurable by absorption photometry.

Dehydrocholic acid also reduced rates of synthesis and mobilization of cholesterol-x-C^{14} in serum, liver, and adrenals, but to a lesser extent than cholic acid (Table II).

Expired CO_2. Exp. A and B. Acetate oxidation rate and acetate pool levels were compared in control and experimental groups by measurement of $C^{14}O_2$ activity. Table III shows that there was no significant difference between controls and treated animals in either experiment.

Discussion. From the reported results, it is clear that dietary cholic acid at the 0.5% level is a very potent inhibitor of both cholesterol synthesis and mobilization (*in vivo*). Also, at this level cholic acid does not cause accumulation of tissue cholesterol, when the diet is cholesterol free. This is due to the fact that the rates of synthesis and disappearance declined simultaneously, and thus

TABLE II. Serum, Liver and Adrenal Cholesterol-x-C^{14} Levels in Normal and in Cholic and Dehydrocholic Acid Treated Rats as a Function of Time.

Exp.	Group	Time, hr	Cholesterol-x-C^{14}, counts/min./mg cholesterol		
			Serum	Liver	Adrenal
A	Control	6	2,180	2,397	829
		24	1,631	1,577	1,026
		48	742	878	810
		120	489	507	453
	Cholic acid treated (.5%)	6	459	803	129
		24	500	571	301
		48	325	483	234
		120	311	357	295
B	Control	6	1,538	1,721	956
		24	910	805	682
		48	753	738	810
		120	525	428	610
	Dehydrocholic acid (.5%)	6	953	1,081	505
		24	732	729	458
		48	570	566	536
		120	427	408	485

TABLE III. Effect of Cholic and Dehydrocholic Acids on Rate of Oxidation of Sodium Acetate-1-C^{14}.

Exp.	Group	Total $C^{14}O_2$, counts/min.	Significance
A	Control	$156.5 \times 10^5 \pm 8.8 \times 10^5$	$P = .65$
	Cholic acid treated (.5%)	$159.7 \times 10^5 \pm 9.8 \times 10^5$	
B	Control	$129.4 \times 10^5 \pm 11.35 \times 10^5$	$P = .10$
	Dehydrocholic acid (.5%)	$146.0 \times 10^5 \pm 12.92 \times 10^5$	

maintained normal tissue cholesterol level. Whether or not the drop in synthetic rate is a primary effect of cholic acid can not be decided from these data. There was a very small but significant increase in liver cholesterol level, and it is well known that accumulated cholesterol will retard the rate of cholesterol synthesis.

The hypercholesterolemia resulting from simultaneous feeding of cholesterol and bile acids, can readily be explained by our results. The data in Table II show that cholic acid, and to a lesser extent dehydrocholic acid, reduced rate of mobilization of serum and liver cholesterol. Pihl has shown that dietary bile acids have little effect on rate of cholesterol absorption at the 0.5% level. Therefore, simultaneous feeding of cholesterol and bile acids must result in cholesterol accumulation.

In agreement with previous studies(1,2) cholic and dehydrocholic acids have similar but quantitatively different effects.

Summary. 1) A 3-week period of feeding a diet containing 0.5% cholic acid did not alter serum and adrenal total cholesterol levels. However, a slight but significant increase in liver total cholesterol was observed. Dietary dehydrocholic acid had no effect on serum, liver or adrenal total cholesterol levels. 2) Cholic acid increased serum bile acid concentration, while dehydrocholic acid had no effect. 3) Dietary cholic acid reduced rates of synthesis and mobilization of cholesterol in both liver and adrenal. Dehydrocholic acid produced similar results but to a lesser degree. 4) Accumulation and mobilization of serum cholesterol were retarded by dietary cholic acid, and slightly by dehydrocholic acid.

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Effects of Gradual Complete Occlusion of Hepatic Veins in Dogs. (24221)

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The basic physiology of portal hypertension and ascites associated with portal cirrhosis is not satisfactorily explained. Proposed experimental methods for producing portal cirrhosis have failed to reproduce a clinical picture of portal hypertension(1-4). Madden *et al.*(5) working with corrosion specimens of the liver postulated that the primary factor in formation of ascites is an obstruction of the outflow tract of the liver, namely, the hepatic veins. This hypothesis appeared to us to be worthy of further experimental verification. After preliminary work on the anatomy of hepatic veins in dogs, we devised a relatively simple procedure for occlusion of these vessels.

Materials and methods. Adult mongrel dogs ranging in weight from 12.5 to 20.0 kg