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Influence of Bile Acids on Cholesterol Metabolism in the Mouse.* (25232)

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Cholic and dehydrocholic acids alter rates of cholesterol synthesis and mobilization in rat liver and adrenal(1,2,3). Since the results of these and other investigations(4,5) have implicated the bile acids in accumulation of dietary cholesterol and in homeostatic control of cholesterol metabolism; cholic, hyodeoxycholic, deoxycholic and lithocholic acids, were selected for this study of cholesterol metabolism in mouse tissues.

Procedure. Seventy Webster strain female albino mice, weighing 18-20 g, were placed on synthetic basal cholesterol free diet (CFD) (6) for 2 weeks, then divided into 7 equal groups. Groups A and F were continued on CFD; Group B received CFD plus 0.5% cholic acid; Groups C and G, CFD plus 0.5% hyodeoxycholic acid; Group D, CFD plus 0.5% deoxycholic acid; and Group E, CFD plus 0.5% lithocholic acid. After receiving these diets *ad lib.* for 3 weeks, all animals were given an intraperitoneal injection of 5 μc sodium acetate-1-C^{14} in saline. Mice in Groups A, B, C, D and E were sacrificed one hour after injection; those in groups F and G, 15 minutes after injection. Liver, small intestine and kidneys were removed for determination of total cholesterol, as previously described (7), and cholesterol-x-C^{14}. The latter was isolated as the digitonide and counted in a Windowless Flow Counter(1).

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Results. The effects of bile acids on cholesterol levels and synthesis rates in mouse liver, small intestine, and kidney are presented in Tables I and II.

Cholic acid produced a 51% increase in total cholesterol level in the liver, a small but significant increase in intestinal cholesterol, and no change of this level in kidney. At the same time there was a striking decrease in the rate of hepatic cholesterol synthesis. Despite

TABLE I. Effect of Bile Acids on Cholesterol Levels in Mouse Tissues.

Group	Total cholesterol, mg/g* tissue		
	Liver	Small intestine	Kidney
A. Control	5.14 ±1.10	2.21 ±.20	4.42 ±.39
B. Cholic acid, .5%	7.76 ±1.74	2.80 ±.18	4.58 ±.42
C. Hyodeoxycholic acid, .5%	2.82 ± .38	2.23 ±.16	4.43 ±.31
D. Deoxycholic acid, .5%	3.88 ±1.16	1.49 ±.54	3.96 ±.28
E. Lithocholic acid, .5%	3.28 ± .75		

Statistical comparison of values:

Groups	Liver	Intestine	Kidney
A and B	P <.01	P <.01	P = .38
A " C	"	P = .80	P = 1.00
A " D	P = .03	P <.01	P <.01
A " E	P <.01		

* Wet wt.

TABLE II. Effect of Bile Acids on Incorporation of Acetate-1-C^{14} into Mouse Tissue Cholesterol.

Group	Activity of cholesterol from 1 g* tissue, counts/min. Liver	Small intestine
A. Control	1,806 ± 508	3,055 ± 587
B. Cholic acid, .5%	900 ± 172	3,244 ± 614
C. Hyodeoxycholic acid, .5%	7,283 ±1,532	5,484 ±1,104
D. Deoxycholic acid, .5%	230 ± 75	
E. Lithocholic acid, .5%	4,065 ±1,700	

Statistical comparison of values:

Groups	Liver	Intestine
A and B	$P < .01$	$P = .52$
A " C	"	$P < .01$
A " D	"	
A " E	"	

* Wet wt.

the small increase of intestinal cholesterol, incorporation of acetate-1-C^{14} into cholesterol was unchanged.

Hyodeoxycholic and lithocholic acids effected significant decreases in hepatic cholesterol levels (45 and 36%), while greatly increasing incorporation of acetate-1-C^{14} into hepatic cholesterol. Hyodeoxycholic acid did not alter levels of intestinal or kidney cholesterol, but appeared to increase the synthesis rate of cholesterol in the small intestine of animals sacrificed one hour after injection. However, this increase in cholesterol-x-C^{14} concentration over control level does not represent a real increase in intestinal synthesis rate, since the cholesterol-x-C^{14} level of the treated animals as compared with the controls was not significantly increased in groups sacrificed 15 minutes after injection (Table III). Thus, the increased cholesterol-x-C^{14} level at the one hour interval originated extra-intestinally. A similar consideration makes it apparent that observed increases in cholesterol-x-C^{14} levels in extra-hepatic tissues of mice sacrificed an hour after injection do not indicate real increases in synthesis rates. On the other hand, in the case of liver, the data at 15 minutes and 1 hour show that there is a real increase in synthesis rate in this organ.

Deoxycholic acid differed from the other bile acids inasmuch as it produced significant decreases in liver, intestine and kidney cholesterol levels, along with a large decrease in hepatic cholesterol synthesis rate. These directly proportional effects on cholesterol levels and synthesis rates indicate that this bile acid does not act by the same mechanism as the other bile acids, and should be further investigated.

These experiments, together with previous investigations(1,2,3), have determined the general effects of 5 dietary bile acids on cholesterol metabolism in mouse or rat. With the exception of deoxycholic acid, the effect of each of these substances on the incorporation rate of acetate-1-C^{14} into liver cholesterol is inversely related to the changes in hepatic cholesterol concentration. These findings suggest that, as in the case of cholic acid(3), the effects of various bile acids on cholesterol synthesis reflect their effects on hepatic cholesterol levels. Inasmuch as some of these substances are representative of the major bile acids produced during cholesterol catabolism, their role in homeostatic control of liver cholesterol metabolism can be considered significant. It must be emphasized that however effective these substances are in altering hepatic cholesterol metabolism, all other tissues investigated, except adrenal(1), were not altered to any great extent. This is especially interesting in the cases of cholic and

TABLE III. Cholesterol-x-C^{14} Levels in Liver and Intestine 15 Minutes and 1 Hour after Injection of Sodium Acetate-1-C^{14}.

Group	Activity of cholesterol from 1 g* tissue, counts/min. Liver 15 min.	Liver 1 hr	Small intestine 15 min.	Small intestine 1 hr
F. Control	2,629 ± 697	1,806 ± 508	3,837 ± 1,196	3,055 ± 587
G. Hyodeoxycholic acid, .5%	6,325 ± 1,040	7,283 ± 1,532	4,412 ± 895	5,484 ± 1,104
Statistical comparison of values:				
Groups F and G	$P < .01$	$P < .01$	$P = .25$	$P < .01$

* Wet wt.

hyodeoxycholic acids, which bring about striking changes in hepatic cholesterol levels and synthesis rates in the mouse, but have little or no effect on small intestine or kidney. It would appear that in the mouse or rat, homeostatic control of cholesterol metabolism in kidney and intestine is more or less independent of hepatic control and of the bile acids influencing cholesterol synthesis in the liver.

Summary. Feeding various bile acids to mice for 3 weeks had the following effects: 1. Cholic acid increased hepatic and intestinal cholesterol levels, but had no effect on kidney cholesterol. It decreased hepatic cholesterol synthesis rates, but had no effect on intestinal synthesis rates. 2. Hyodeoxycholic and lithocholic acids decreased liver cholesterol levels. Hyodeoxycholic acid had no effect on intestine and kidney cholesterol, or on intestinal cholesterol-x-C^{14}. Both acids effected large increases in hepatic cholesterol synthesis. 3. Deoxycholic acid significantly decreased liver, small intestine and kidney cholesterol levels. It also decreased hepatic cholesterol synthesis. 4. Results of this study suggest that homeostatic control of cholesterol metabolism in tissues other than liver is independent of hepatic control.

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Relative Abundance of Vasopressin and Corticotrophin-Releasing Factor in Neurohypophysial Extracts.* (25233)

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In our previous experiments using rats with median eminence lesions to assay adrenocorticotrophin (ACTH)-releasing activity, the ACTH-releasing potency of posterior pituitary preparations was accounted for by their vasopressin content(1,2). Saffran and Schally (3) and later Guillemin and co-workers(4) reported, on the other hand, that extracts of posterior lobe of the pituitary gland contain a corticotrophin-releasing factor (CRF), distinct from vasopressin, which releases ACTH from pituitaries, *in vitro*. Attempts to demonstrate activity of this CRF of Guillemin *in vivo* were unsuccessful(1) using rats with hypothalamic lesions. Recently, Royce and Sayers(5) reported the presence of a CRF distinct from vasopressin in extracts of calf stalk-median eminence tissue, assaying the activity in rats with median eminence lesions. This observation prompted a reinvestigation of the ACTH-releasing activity of various brain extracts from hypothalamus and other regions, utilizing rats with lesions to assess ACTH-releasing activity.

Methods. Preparation of animals. Hypothalamic lesions were made in the median eminence of the tuber cinereum using methods previously reported(1) except that the rats were injected intramuscularly with 60,000 U crystalline procaine penicillin G at operation. Rats drinking at least 100 ml of water during the first 24 hours post-operatively were selected for the assay procedure. This assay of ACTH-releasing activity was performed 48 hours after placing the lesions, since at that time such rats have been shown to be unresponsive to a variety of non-specific stimuli (1-2). Proper selection of coordinates can

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