Effect of Cholic and Hyodeoxycholic Acids on Metabolism of Exogenous Cholesterol in Mice.* (25529)

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Among several bile acids investigated(1), cholic and hyodeoxycholic acids had opposing effects on liver cholesterol metabolism in mice. Cholic acid increased liver cholesterol levels and decreased cholesterol synthesis; hyodeoxycholic acid decreased liver cholesterol levels, and greatly increased the synthesis rate. Investigations have shown that the influence of cholic acid on accumulation of cholesterol of exogenous origin is due to 2 factors: increased absorption and decreased cholesterol mobilization(2,3,4). It seemed possible that hyodeoxycholic acid might be of value in preventing accumulation of exogenous cholesterol, since its effects on cholesterol synthesis and levels appear to be opposite to those of cholic acid. We have therefore tested the effect of hyodeoxycholic acid on mouse liver cholesterol levels under various dietary conditions.

Methods. Eighty Webster strain female albino mice, weighing 20-25 g, were placed on a cholesterol free diet (CFD), having the following composition: vitamin-free casein, 25%; sucrose, 55%; non-nutritive bulk (alphacel†), 16%; and Phillips Hart salt mixture,† 4%. This was supplemented with following quantities of vitamins, expressed as mg/10 kg: thiamin hydrochloride, 40.4; riboflavin, 60.8; calcium pantothenate, 151.0; niacin, 1,008; pyridoxine hydrochloride, 40.4; 2-methyl-1,4-naphthoquinone, 5.12; alpha tocopherol, 30.2; folic acid, 20.3; biotin, 2.02; choline chloride, 20,200; p-aminobenzoic acid, 202; inositol, 10,100; Vit. A, 1.25 x 10^6 units. After one-week observation, mice were divided into 8 groups and placed on these diets *ad lib.*:

Group	Diet
A	CFD
B	CFD + cholic acid (CA), .5%
C	CFD + hyodeoxycholic acid (HDCA), .5%
D	CFD + cholesterol, 1%
E	*Idem* + CA, .5%
F	" + HDCA, .5%
G	" + CA, .5% + HDCA, .5%
H	" + CA, .5% + HDCA, .25%

At end of 4-week experimental period, all mice were sacrificed, livers removed and analyzed for total cholesterol(5). Stomachs and intestines were removed and cleared of all ingested material. The combined carcass, blood, stomach, and intestine were hydrolyzed by refluxing for 3 hours in 5% alcoholic potassium hydroxide. Aliquots of the hydrolysate were used for total cholesterol determination (5). Blood cholesterol levels were determined according to Sperry and Webb(6). Expired CO_2 was collected by means of Ascarite traps and determined gravimetrically.

Results. Table I presents results of this experiment. As expected, supplementing CFD with 0.5% cholic acid (Group B) caused a 51% increase in liver total cholesterol, whereas supplementation with hyodeoxycholic acid (Group C) caused a 58% decrease. Blood total cholesterol levels were 25% lower in the hyodeoxycholic acid treated animals (Group C, 74.1 $\pm$ 9.0 mg %) than in the controls (Group A, 98.3 $\pm$ 12.9 mg %). Supplementing CFD with 1.0% cholesterol (Group D) failed to increase either liver or carcass total cholesterol.

However, when 0.5% cholic acid (Group E) as well as cholesterol was added to the diet, there were significant increases in both liver and carcass total cholesterol (240% and 30%). On the other hand, addition of hyodeoxycholic acid (Group F) to the diet containing cholesterol, brought about a reduction in total liver cholesterol but had no effect on total carcass cholesterol.

A comparison of results for Group E with those for Groups G and H, indicates that adding hyodeoxycholic acid to the combination of 1% cholesterol and 0.5% cholic acid pre-

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† Nutritional Biochemicals Corp., Cleveland, O.

TABLE I. Effect of Cholesterol and Bile Acids on Liver and Carcass Cholesterol Levels.

	Group	Total cholesterol (mg/g) Liver	Carcass*	Avg wt (g) Liver	Body
A	Control	7.30 ± .80	2.37 ±.44	2.35 ±.39	27.5 ± 2.2
B	Cholic acid (CA), .5%	11.0 ±2.48		2.08 ±.26	25.1 ± 2.2
C	Hyodeoxycholic acid (HDCA), .5%	3.05 ± .60	2.18 ±.25	2.23 ±.30	28.7 ± 1.3
D	Cholesterol, 1%	7.33 ± .97	2.74 ±.63	2.47 ±.31	27.9 ± 2.1
E	*Idem* + CA, .5%	24.7 ±6.30	3.08 ±.43	2.34 ±.37	25.8 ± 3.6
F	" + HDCA, .5%	3.33 ± .66	2.34 ±.25	2.17 ±.21	27.1 ± 1.4
G	" + CA, .5% + HDCA, .5%	6.21 ±2.10	2.36 ±.38	2.07 ±.33	24.8 ± 2.5
H	" + CA, .5% + HDCA, .25%	8.28 ±1.03	2.82 ±.27	1.98 ±.47	24.2 ± 4.1

Statistical comparison of values:

Groups	Liver	Carcass	Liver wt	Body wt
A and B	$P < .01$		$P = .08$	$P = .03$
A " C	"	$P = .28$		
A " D	$P = 1.00$	$P = .16$		
A " E	$P < .01$	$P < .01$	$P = 1.00$	$P = .27$
A " F	"	$P = 1.00$		
A " G	$P = .18$	$P = 1.00$	$P < .01$	$P = .05$
A " H	$P = .05$	$P = .01$	$P = .07$	"
E " G	$P < .01$	$P < .01$		
E " H	"	$P = .12$		
G " H	$P = .05$	$P = .01$		

* Without liver.

vents the usual accumulation of liver and carcass cholesterol. At 0.5% level (Group G), hyodeoxycholic acid prevented the accumulation of 45 mg of cholesterol per mouse liver, and 19 mg of cholesterol per carcass. While the effect at the 0.25% level (Group H) was smaller, it was still significant.

It should be noted that a diet supplemented with cholic acid caused significant decreases in liver and body weight, whereas hyodeoxycholic acid did not affect either liver or body weight.

Control of accumulation of cholesterol from both exogenous and endogenous sources is a subject of considerable interest. It has been shown(2,4) that cholesterol accumulation caused by cholic acid and its conjugates is due not only to an increase in cholesterol absorption but also to a decrease in rate of cholesterol mobilization. The present results show that in mice hyodeoxycholic acid prevents the build-up of tissue cholesterol of exogenous as well as of endogenous origin.

There are a number of possible explanations for this action of hyodeoxycholic acid: It may (a) prevent absorption of cholesterol from the intestinal lumen; (b) increase rate of hepatic cholesterol degradation and mobilization; (c) cause redistribution of liver cholesterol among other tissues; or (d) increase basal metabolic rate by affecting the thyroid gland.

Mechanism *c* does not seem possible because there was a decrease rather than an increase in carcass cholesterol at the same time that hyodeoxycholic acid prevented liver cholesterol accumulation. Mechanism *d* is eliminated because the metabolic rates of control (Group A) and hyodeoxycholic acid treated (Group C) animals did not differ significantly. CO_2 production in the 2 groups was: Group A, 1.52 ± 0.10 mg/hr/cm^2; and Group C, 1.63 ± 0.23 mg/hr/cm^2. The present experiment does not provide the necessary data to decide between mechanisms *a* and *b*.

Summary. In the female mouse fed a cholesterol-free diet: (1) supplement of 1% cholesterol did not alter liver or carcass cholesterol levels; (2) 1% cholesterol plus 0.5% cholic acid brought about large increases in liver and carcass cholesterol levels; (3) hyodeoxycholic acid reversed the effect of cholic acid and prevented cholesterol accumulation.

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Effect of Ethanol on Cytological Changes Induced by Salt Load in Nucleus Supraopticus of Rat.* (25530)

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It is generally agreed that posterior pituitary hormones are formed in the hypothalamus and transported *via* supraoptico-hypophyseal tract to the posterior pituitary lobe bound to a carrier, neurosecretory material (1). Although this hypothesis of neurosecretion is widely accepted no agreement has been reached as to site of formation of the peptide hormones or to the mechanism of their release. According to Olivecrona(2), formation of oxytocin is primarily located in the paraventricular nucleus and that of antidiuretic hormone (ADH) in the supraoptic nucleus. Conspicuous changes in ganglion cells of the supraoptic nucleus after procedures which increase blood tonicity were first noted by Ortmann(3), and later confirmed by other investigators(4). According to Ortmann(3) cytological changes in cells of nucleus supraopticus after osmotic stress with hypertonic saline are comparable with those found by Hydén(5) in nerve cells activated by other stimuli. It was further suggested that these changes reflect increase of cell activity and hence production of antidiuretic hormone. The diuretic action of ethanol has been explained as due to inhibition of the supraoptico-hypophyseal system(6,7). Kleeman *et al.*(8) in studies on ethanol diuresis in human subjects found that ethanol prevented or minimized the fall of urine flow and free water clearance that characteristically follows administration of hypertonic solutions of sodium chloride. In the present investigation the cytological changes of the supraoptic nucleus of rats induced by hypertonic salt solution were compared with those of rats receiving ethanol in addition to hypertonic salt solution.

Material and methods. White male rats weighing 150 to 170 g were used. The animals were divided into 4 groups of 7 rats. Twelve hours after withdrawal of food, rats in Group I received an intraperitoneal injection of 6 ml of water, Group II, 6 ml of 10% (w/v) solution of ethanol, Group III, 6 ml of 5% NaCl solution and Group IV, 6 ml of 5% NaCl solution containing 10% ethanol. Each rat was placed in metabolic cage and urine collected 24 hours. Rats used for histological studies were divided into 3 groups of 4 rats. 5 ml of 10% ethanol solution was administered by stomach tube twice a day during 5 days to the control group. The second group received an equal amount of 2.5% NaCl solution and the third group a solution containing 10% ethanol in 2.5% NaCl. On 6th day animals were killed by decapitation. The hypothalamic region was fixed for 24 hours in Bouin's fluid, treated with alcohol and embedded in paraffin. Transverse sections of 3 to 5 μ were cut through the supraoptic nucleus and stained according to Gomori's chromhematoxylin method.

Results. Table I shows total urine excre-

TABLE I. Total Urine Excreted in ml during 24 Hours in 4 Experimental Groups of Rats.

	Group I	Group II	Group III	Group IV
	Water	Ethanol	NaCl	NaCl + ethanol
	7.5	9.5	4.5	9.0
	7.0	9.0	5.0	8.0
	6.5	10.0	6.8	8.5
	4.5	7.5	3.5	7.0
	8.0	9.0	5.6	6.5
	6.0	8.0	5.5	7.5
	7.0	11.0	6.5	7.0
Mean	6.64	9.21	5.34	7.64

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