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Comparative Effects of Bile Acids on Intestinal Absorption of Cholesterol.* (25008)

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Schoenheimer and co-workers reported the first critical experiments on facilitatory effect of bile salts on cholesterol absorption in various animals(1-3). Using serum or liver cholesterol levels as indicators of quantitative differences between bile acids, these workers found that glycocholate had the greatest effect in enhancing cholesterol absorption, while cholic acid and desoxycholic acid were less effective(2,3). Member *et al.*(4) reported that cholate or glycocholate fed with cholesterol to rabbits increased blood and aorta cholesterol levels, while dehydrocholate, hyodesoxycholate, and desoxycholate were ineffective. In chronic feeding experiments with rats, Swell and co-workers(5) found that blood cholesterol levels were elevated by inclusion of cholate or taurocholate in high fat-high cholesterol diets; desoxycholate was less effective, while dehydrocholate was without noticeable effect. Correlation of these data with *in vitro* studies on comparative effects of different bile salts on cholesterol esterase activity(6) led to the suggestion that the observed effects of dietary bile salts were due to their requirement as cofactors for cholesterol esterase activity. Another explanation is that due to hydrotropic properties of these substances(7), dispersion of cholesterol and its tranfer into the mucosa is increased. Recently, Swell and coworkers(8) demonstrated that bile salts are involved in passage of cholesterol from the intestinal lumen into the mucosa. Further, these authors suggested that a specific type of bile salt was involved, since dehydrocholate was ineffective in promoting transfer of cholesterol into the intestinal mucosa, while taurocholate increased both mucosal and lymph cholesterol levels(8). Other workers have suggested that accumulation of cholesterol in blood and liver when bile salts are fed is related to depressed rate of cholesterol catabolism or excretion(9,10). The purpose of the present investigation was to determine directly, using changes in lymph cholesterol levels, the comparative effects of various bile salts on intestinal absorption of cholesterol in the rat.

Methods and materials. Male rats of Carworth Farms strain with thoracic duct lymph fistula were prepared and handled as described previously(11). Following a fasting period of 18-24 hours, the animals were given 3 ml of aqueous test emulsion intragastrically (11). Control and test emulsions contained 50 mg of albumin, 50 mg of cholesterol and 288 mg of oleic acid/3 ml of 0.9% saline. In addition, the experimental emulsions contained 4 molar equivalents of one of 7 different bile acids/mole of cholesterol fed. Commercial samples of lithocholic acid, deoxycholic acid, dehydrocholic acid, sodium taurocholate and sodium glycocholate (Nutritional Biochemicals) were employed. In addition, highly purified samples of cholanic acid,† cholic acid‡ and synthetic

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† Mann Research Lab., M.P. 163.5°C; sp. rot., + 21°.

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TABLE I. Effect of Various Bile Acids on Absorption of Cholesterol in 9 Groups of Lymph-Fistula Rats.

Additions	No. of rats	Avg lymph vol/24 hr, ml	Lymph cholesterol/24 hr — Total, mg	Free, mg	Ester, %
None*	4	57 ± 9	21 ± 2	5 ± .2	76 ± 2
Cholanic acid	3	53 ± 5	20 ± 2	5 ± .3	74 ± 1
Lithocholic acid (3-hydroxycholanic acid)	4	72 ± 6	20 ± 2	6 ± .8	72 ± 3
Deoxycholic acid (3,12-dihydroxycholanic acid)	4	51 ± 5	24 ± 2	6 ± .6	77 ± 1
Cholic acid (3,7,12-trihydroxycholanic acid)	5	118 ± 29	40 ± 3†	7 ± .6†	82 ± 1†
Dehydrocholic acid (3,7,12-triketocholanic acid)	5	69 ± 12	21 ± 1	6 ± .4	74 ± 0
Glycocholate	4	132 ± 22†	32 ± 3†	7 ± .2†	77 ± 2
Taurocholate	5	94 ± 24	33 ± 2†	7 ± .2†	80 ± 2†
Synthetic taurocholate	5	99 ± 23	33 ± 1†	6 ± .5	82 ± 1†

* Basic emulsion for all groups contained 50 mg cholesterol, 50 mg albumin, and 288 mg oleic acid/3 ml of .9% saline/rat(15).

† $p < .05$ for difference between experimental and control group.

sodium taurocholate(12) were also used. Lymph was collected for 24 hours after administration of test emulsions and frozen until analyzed for total and free cholesterol(13). Values for esterified cholesterol were obtained by difference. There were 3-5 animals/group, and apparent differences between control and experimental groups were analyzed for significance by the t-method of Fisher(14). Probability levels of $p<0.05$ were considered significant.

Results. Previous investigations of effects of different bile acids on cholesterol absorption have been long-term in nature and have employed variations in blood and liver cholesterol levels as indicators of cholesterol absorption from the intestine. In these approaches there is the possibility that increases in tissue sterol levels are due to effects on cholesterol metabolism in liver or other tissues (9,10) and do not specifically measure intestinal absorption. The present approach is more suitable for studying the effect of bile acids directly on cholesterol absorption since thoracic duct lymph is the primary transport route for absorbed cholesterol(16,17); also a single gastric feeding has been employed eliminating any long-term effects of bile acids.

The results (Table I) show that cholanic acid, lithocholic acid (3-hydroxycholanic acid) and deoxycholic acid (3,12-dihydroxycholanic acid) have no direct effect on absorption of cholesterol from the intestinal tract. These results are in general similar to those of earlier studies based on measurements of blood and liver cholesterol levels. However, those technics have generally shown a significant effect by deoxycholic acid; this was not found in our study. Thus, long-term feeding of deoxycholic acid in diets containing cholesterol produces changes in tissue cholesterol levels which are not due directly to increased absorption of dietary cholesterol. Administration of cholic acid (3,7,12-trihydroxycholanic acid) with cholesterol and oleic acid gave an increase in total lymph cholesterol of 19 mg/24 hours, which was equivalent to 38% of that fed. This facilitatory effect of cholic acid on cholesterol absorption was greater than with other bile acids tested. Thus, it appears that presence of 3 hydroxyl groups on the bile acid is necessary for the activity in promoting cholesterol absorption, since removal of even one, as in deoxycholic acid, results in a loss of this effect. Also, dehydrocholic acid, containing 3 keto groups, had no effect in promoting cholesterol absorption.

Comparison of effects of taurocholate or glycocholate on cholesterol absorption with that of cholic acid shows that esterifying the free carboxyl group of the bile acid depresses its effect on cholesterol absorption. The type of amino acid conjugated to cholate is not critical since both taurocholate and glycocholate had the same quantitative effect on

absorption of cholesterol.

Differences in lymph free cholesterol between control and experimental groups were insignificant except for those fed cholic acid, glycocholate, and commercial taurocholate. In these groups where cholesterol absorption occurred, values for free cholesterol were greater than in the control group; this relationship has been discussed(11). The data clearly demonstrate that certain bile salts have a direct effect on intestinal absorption of cholesterol in addition to the reported effect of these substances on hepatic metabolism of cholesterol(9,10).

Summary. 1. Effect of various bile acids on intestinal absorption of dietary cholesterol has been investigated in lymph-fistula rats. 2. Cholanic, lithocholic, and deoxycholic acids, with none, one and 2 hydroxyl radicals, respectively, produced no increase in lymph cholesterol over the control group. 3. Conjugated bile salts, glycocholate and taurocholate gave comparable significant elevations in total lymph cholesterol/24 hours. 4. Cholic acid with 3 free hydroxyl groups and an unconjugated carboxyl radical was the most effective bile acid in promoting cholesterol absorption from the intestine.

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Fluorescent-Antibody Technic for Serodiagnosis of *Trypanosoma cruzi* Infection.* (25009)

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Since Coons and his colleagues first reported the immuno-specific staining of tissue with fluorescein-conjugated globulin(1), numerous applications of the technic have been found. It was shown that fluorescein-labeled antibody not only provided a means of demonstrating localized antigen in tissues, but could be used to detect specific antibody developed during the course of infection with certain micro-organisms, *e.g.*, *Toxoplasma gondii*(2) and *Treponema pallidum*(3). The present report describes a fluorescent-antibody test developed for serodiagnosis of American trypanosomiasis (Chagas' disease), and gives an improved method for purifying antibody prior to conjugation with fluorescein. The procedure was evaluated in tests with homologous and heterologous sera and the results compared with those obtained in complement fixation tests.

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Materials and methods. The indirect technic, using a labeled antihuman antibody, was