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Effect of Partial Ileal Bypass on Cholesterol and Bile Acid Metabolism in Rats

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In order to clarify the effect of ileal bypass on cholesterol and bile acid metabolism, partial (20 cm) ileal bypass rats were fed a 2% cholesterol supplemented diet for a week after 4 weeks of the operation. The serum and liver cholesterol and phospholipid levels, biliary cholesterol, phospholipid and bile acid secretions, and fecal cholesterol, coprostanol and bile acid excretions were examined. The serum cholesterol level in ileal bypass rats was lower than in normal rats and no hypercholesterolemia was brought about in ileal bypass rats by feeding them the cholesterol diet. The liver cholesterol level increased by feeding the cholesterol diet even in ileal bypass rats but the increase was far less than that in normal rats (22% versus 57%). Biliary bile acid secretion decreased and fecal bile acid excretion increased markedly in ileal bypass rats. Deoxycholic acid increased remarkably in both bile and feces and resulted in an increase in the ratio of the sum of bile acids derived from cholic acid over the sum of bile acids derived from chenodeoxycholic acid (CA/CDCA ratio) in the feces. These results suggest that the absorption of bile acids is impaired, the pool size of bile acids decreases and the hepatic synthesis of bile acids, especially that of cholic acid, increases in ileal bypass rats. As a result, cholesterol feeding to ileal bypass rats produces neither hypercholesterolemia nor a further increase in bile acid synthesis.

Key words: biliary lipids; fecal bile acids; fecal sterols; ileal bypass rat; serum and liver lipids

Bile acids are synthesized in the liver and secreted into the bile. The bile acids excreted in the duodenum form micelles with dietary lipids and enhance intestinal absorption of the lipids. The bile acids themselves are absorbed by the active transport mechanism confined to the ileum and by the passive transport mechanism at any part of the gastrointestinal tract (Dietschy, 1968; Schiff et al., 1972). The bile acids absorbed return to the liver through the portal vein and are excreted again into the bile, forming the so-called enterohepatic circulation (Carey and Duane, 1994). Most of the bile acids secreted into the duodenum are absorbed from the intestine, but part of them, less than 5% of the bile acid pool, is excreted into the feces. In a steady state, the amounts of bile acids daily excreted into the feces almost correspond to the amounts of bile acids synthesized daily in the liver.

Bile acids synthesized in the liver are cholic acid and chenodeoxycholic acid in many animal species including humans. In rats and mice, chenodeoxycholic acid is further converted to α- and β-muricholic acids in the liver (Botham and Boyd, 1983; Ogura and Ayaki, 1987). These primary bile acids are transformed into various secondary bile acids during enterohepatic circulation (Macdonald et al., 1983; Groh et al., 1993). Cholic acid is transformed into deoxycholic acid and 3α,12α-dihydroxy-7-oxo-5β-

Abbreviations: CA/CDCA ratio, ratio of the sum of bile acids derived from cholic acid over the sum of bile acids derived from chenodeoxycholic acid; C/P, cholesterol/phospholipid

cholanoic acid; chenodeoxycholic acid, into lithocholic acid and 3α-hydroxy-7-oxo-5β-cholanoic acid; and α- and β-muricholic acids, into ω-muricholic acid, hyodeoxycholic acid and 3α-hydroxy-6-oxo-5β-cholanoic acid.

One important biological role of bile acids is to enhance absorption of lipids including cholesterol, but the effect differs with individual bile acids (Uchida et al., 1980; Reynier et al., 1981). When rats were fed a cholesterol supplemented diet with bile acids, the serum and liver cholesterol levels were markedly increased by a simultaneous feeding of cholic and deoxycholic acids, but not by that of chenodeoxycholic, lithocholic, hyodeoxycholic and ursodeoxycholic acids (Uchida et al., 1980, 1983). Furthermore, when the effect of bile acids on enhancement of cholesterol absorption was examined in situ by a rat intestinal loop method, the cholesterol absorption was greater with taurocholic acid than with taurochenodeoxycholic acid or taurodeoxycholic acid (about half of the effect of taurocholic acid), and almost no effect with tauro-β-muricholic acid or tauroursodeoxycholic acid (Uchida et al., 1990).

The primary bile acids synthesized in the liver are cholic and chenodeoxycholic acids, but since chenodeoxycholic acid is easily converted to β-muricholic acid in the rat liver, the main bile acids found in rats are cholic acid and β-muricholic acid, and these two comprise over 80% of the bile acid pool (Uchida et al., 1978). Cholic acid enhances cholesterol absorption, while β-muricholic acid does only to a small degree. Therefore, the composition ratio of both bile acids (CA/CDCA ratio), is an important factor in cholesterol absorption.

On the other hand, ileal resection or ileal bypass operation is adopted clinically to prevent obesity or hypercholesterolemia (Buchwald et al., 1974). Since about 60% of the bile acids in the intestine is absorbed from the ileum during the enterohepatic circulation of bile acids (Dietschy, 1968; Kanamura, 1982), dysfunction of the ileum will cause decreases in bile acid absorption and bile acid pool size, resulting in a decrease in cholesterol absorption. In the present series of experiments, we examined bile acid metabolism and serum and liver cholesterol levels in ileal bypass rats kept on an ordinary diet and a 2% cholesterol supplemented diet. We adopted a 2% cholesterol concentration according to our previous experiments (Uchia et al., 1970, 1977).

Materials and Methods

Animal treatments

Wistar strain male rats weighing about 300 g (10–12 weeks old) were kept in an air-conditioned room (25 ± 1˚C, 50–60% humidity) lighted 12 h a day (0800–2000) and maintained on a commercial stock diet (Japan CLEA CA-1, Tokyo, Japan). The cholesterol diet was prepared by adding 2% (w/w) powdered cholesterol to the basal diet, and fed to rats for a week after 4 weeks of the operation.

The rats were laparotomized along the median line under sodium pentobarbital anesthesia (50 mg/kg, intraperitoneally) and the intestine was cut at a level of about 20 cm from the ileum end. The proximal intestine was anastomosed to the cecum and the distal end was closed (Buchwald et al., 1974). A length of 20 cm corresponds to about 1/4 of the intestine (from Treitz's ligament toward the ileum end).

Rats were individually caged during the period of experimentation and 2-day feces specimens were collected prior to sacrifice as described previously (Uchida et al., 1977). Five weeks after the ileal bypass operation, and one week after beginning of the cholesterol diet, the rats were cannulated of their bile ducts with PE-10 polyethylene tubings to collect the bile for 30 min under sodium pentobarbital anesthesia (50 mg/kg, intraperitoneally). During the time of bile collection, the rectal temperature was maintained at 36–37˚C using an electric warm plate. The blood was then withdrawn by heart puncture and the liver was removed for lipid determination. Bile was collected between 0900 and 1100 to avoid any variation due to the circadian rhythm.

Serum and liver lipid determination

The serum was separated by centrifugation at 3000 revolutions per minute for 15 min after allowing the blood to stand for at least 30 min at room temperature. A portion of the largest lobus of the liver (*lobus sinistra externa*) was homogenized with 9 volumes of water. The serum and the liver homogenate were extracted in 10 volumes of ethanol by refluxing for 20 min at 90–95˚C. Total cholesterol was determined with portions of the serum and liver lipid extracts as previously reported (Uchida et al., 1965). Phospholipids were determined by the methods of Gomori (1942).

Biliary lipid determination

The bile was added to 20 volumes of ethanol, brought to boil once for several minutes, cooled down to room temperature, and then filtered. An aliquot of the filtrate was evaporated to dryness under a stream of nitrogen and the residue was hydrolyzed at 120˚C for 6 h in 1.25 N sodium hydroxide solution. Sterols were extracted with diethylether, the reaction mixture was acidified with 2 N hydrochloric acid and then the bile acids were extracted with diethylether. The bile acids were methylated with freshly prepared diazomethane and then trifluoroacetylated with trifluoroacetic anhydride. The bile acid derivatives were quantified by gas-liquid chromatography with a 1% QF-1 column and cholesterol with a 1% SE-30 column (Uchida et al., 1970, 1977). Biliary phospholipids were determined by the methods of Gomori (1942).

Lithogenic index was calculated by the formula reported by Thomas and Hofmann (1973), based on the equation of Admirand and Small (1968).

Fecal sterol and bile acid determination

The feces were dried and powdered, according to the procedures described previously (Uchida et al., 1977). An aliquot of the pulverized feces was extracted with 20 volumes of boiling ethanol for 1 h and filtered after cooling down to room temperature. The extraction procedures were repeated three times. The combined filtrate was evaporated to dryness under reduced pressure, and the residue was hydrolyzed at 120˚C for 6 h in 1.25 N sodium hydroxide solution. Sterols were extracted with diethylether, the reaction mixture was acidified with 2 N hydrochloric acid and then bile acids were extracted with diethylether. The methylated and trifluoroacetylated bile acids were quantified by gas-liquid chromatography with a 1% QF-1 column and sterols with a 1% SE-30 column (Uchida et al., 1977; Kinugasa et al., 1981).

Statistical analysis

Data are expressed as mean ± SE. The difference between the means of variables was calculated by Student's *t*-test. Values of $P < 0.05$ were considered as statistically significant.

Results

Changes in body weight, liver weight, serum and liver lipid levels, and diet intake are given in Table 1. No significant change was found in body weight, liver weight or diet intake in ileal bypass rats fed either ordinary diet or the cholesterol diet, compared with normal rats.

The serum cholesterol and phospholipid levels decreased in ileal bypass rats and did not increase even while being fed the cholesterol diet. Therefore, these lipid levels and the cholesterol/phospholipid ratio (C/P ratio) in the cholesterol diet fed ileal bypass rats were lower than those in the corresponding normal rats. The liver cholesterol level increased after being fed the cholesterol diet, but the level in ileal bypass rats was lower than that in normal rats. The liver phospholipid levels were almost the same in all groups.

Data on bile flow, biliary secretions of cholesterol, phospholipid and bile acids, feces dry weight and fecal excretions of cholesterol, coprostanol and bile acids are given in Table 2. No change was found in the bile flow and feces dry weight in ileal bypass rats. A slight decrease in biliary cholesterol secretion was sta-

Table 1. Effects of ileal bypass on serum and liver lipid levels in rats

	Normal rats		Ileal bypass rats	
	Ordinary diet	Cholesterol diet	Ordinary diet	Cholesterol diet
Number of rats	4	3	4	3
Body weight (g)	445 ± 15.0	426 ± 25.0	436 ± 11.9	424 ± 8.1
Serum cholesterol (mg/dL)	75 ± 0.8	100 ± 5.4*	60 ± 3.6*	64 ± 0.4**
Phospholipids (mg/dL)	137 ± 3.3	133 ± 11.3	99 ± 6.6*	100 ± 3.1**
C/P ratio	0.55 ± 0.010	0.75 ± 0.023*	0.60 ± 0.020	0.64 ± 0.017**
Liver weight (g)	3.56 ± 0.289	3.81 ± 0.112	3.19 ± 0.113	3.51 ± 0.123
Cholesterol (mg/g)	3.23 ± 0.138	5.08 ± 0.216*	3.26 ± 0.152	3.97 ± 0.216**,***
Phospholipids (mg/g)	41.6 ± 4.02	42.1 ± 1.28	44.7 ± 0.86	44.3 ± 3.10
Diet intake (g/day/rat)	17.5 ± 0.50	17.2 ± 1.09	19.8 ± 1.42	16.8 ± 1.64

Data, mean ± SE.
C/P, cholesterol/phospholipid.
*Statistically significant compared with normal rats on the ordinary diet ($P < 0.05$).
**Statistically significant compared with normal rats on the cholesterol diet ($P < 0.05$).
***Statistically significant compared with ileal bypass rats on the ordinary diet ($P < 0.05$).

tistically significant in ileal bypass rats on the ordinary diet but not in those on the cholesterol diet. However, a marked decrease in biliary bile acid secretion was found in ileal bypass rats kept on the ordinary and cholesterol diets. The decreases attained about 65% and 52%, respectively.

The lithogenic indexes were not affected by cholesterol feeding, but increased slightly in ileal bypass rats (0.13 ± 0.012% versus 0.20 ± 0.018%; combined data in normal and ileal bypass rats in both diet groups).

The fecal excretion of cholesterol, but not of coprostanol, increased in ileal bypass rats on the ordinary diet, but the increase was obscure after being fed the cholesterol diet. Cholesterol feeding increased the fecal excretion of not only cholesterol but also coprostanol in both normal and ileal bypass rats.

The fecal bile acid excretion was markedly increased in ileal bypass rats. Feeding of the cholesterol diet increased the fecal bile acid excretion in normal rats, but caused no further increase in ileal bypass rats. The level in ileal bypass rats was almost 4-fold that in normal rats kept on the ordinary diet, and about twice that in normal rats on the cholesterol diet.

Changes in biliary bile acid composition are given in Table 3. When normal rats were fed the cholesterol diet, an increase in chenode-

Table 2. Effects of ileal bypass on biliary and fecal lipid levels in rats

	Normal rats		Ileal bypass rats	
	Ordinary diet	Cholesterol diet	Ordary diet	Cholesterol diet
Bile flow (mL/h/rat)	1.38 ± 0.254	1.88 ± 0.111	1.18 ± 0.074	1.37 ± 0.223
Cholesterol (mg/h/rat)	0.23 ± 0.003	0.24 ± 0.001	0.17 ± 0.016*	0.19 ± 0.034
Phospholipids (mg/h/rat)	8.03 ± 0.854	8.94 ± 0.777	5.96 ± 0.415	7.30 ± 1.240
Bile acids (mg/h/rat)	17.61 ± 4.156	16.95 ± 2.898	6.24 ± 0.618*	7.95 ± 1.490**
Lithogenic index (%)	0.13 ± 0.016	0.13 ± 0.023	0.21 ± 0.022*	0.19 ± 0.035
Feces dry weight (g/d/rat)	4.26 ± 0.201	4.38 ± 0.226	4.99 ± 0.372	4.67 ± 0.597
Cholesterol (mg/d/rat)	2.84 ± 0.303	192.5 ± 11.80*	5.68 ± 0.496*	187.1 ± 43.80***
Coprostanol (mg/d/rat)	3.56 ± 0.456	55.7 ± 1.77*	4.05 ± 0.376	51.7 ± 9.69***
Bile acids (mg/d/rat)	11.46 ± 0.742	24.11 ± 0.803*	45.47 ± 5.904*	45.43 ± 7.359**

Data, mean ± SE.
*Statistically significant compared with normal rats on the ordinary diet ($P < 0.05$).
**Statistically significant compared with normal rats on the cholesterol diet ($P < 0.05$).
***Statistically significant compared with ileal bypass rats on the ordinary diet ($P < 0.05$).

Table 3. Effects of ileal bypass on bilary bile acid composition (%) in rats

	Normal rats		Ileal bypass rats	
	Ordinary diet	Cholesterol diet	Ordinary diet	Cholesterol diet
Lithocholic acid	0.4 ± 0.14	1.1 ± 0.22	1.3 ± 0.22*	0.8 ± 0.03
Deoxycholic acid	2.1 ± 0.36	2.9 ± 0.52	33.9 ± 0.88*	22.9 ± 0.20**,***
α-Muricholic acid	3.2 ± 0.93	8.6 ± 2.14	2.9 ± 0.80	4.8 ± 0.38
Chenodeoxycholic acid	3.5 ± 0.74	6.7 ± 0.54*	10.9 ± 2.23*	10.9 ± 2.09
Hyodeoxycholic acid	1.6 ± 0.35	4.9 ± 2.35	1.5 ± 0.47	3.3 ± 1.79
Ursocholic acid	10.6 ± 3.87	7.2 ± 0.82	1.6 ± 0.54*	3.2 ± 1.92
Cholic acid + β-Muricholic acid¶	66.0 ± 7.79	51.2 ± 1.91	42.9 ± 3.03*	47.3 ± 0.92
Oxo-bile acids	10.8 ± 3.35	11.2 ± 2.06	2.5 ± 0.29	4.9 ± 2.09**
Others	2.7 ± 0.47	5.7 ± 1.33	2.5 ± 0.37	1.9 ± 0.23

Mean ± SE.
*Statistically significant compared with normal rats on the ordinary diet ($P < 0.05$).
**Statistically significant compared with normal rats on the cholesterol diet ($P < 0.05$).
***Statistically significant compared with ileal bypass rats on the ordinary diet ($P < 0.05$).
¶Cholic acid and β-muricholic acid were not separable under the present experimental conditions.

oxycholic acid was observed, but no significant change was detected in the other bile acid fractions. Ileal bypass rats showed a marked increase in deoxycholic acid and chenodeoxycholic acid, and a concomitant decrease in the fraction of cholic acid plus β-muricholic acid, these bile acids were not separable under the present experimental conditions. When ileal bypass rats were fed the cholesterol diet, deoxycholic acid decreased but the level was far higher than the corresponding normal rats.

Changes in fecal bile acid composition are given in Table 4. Deoxycholic acid markedly increased, and β- and ω-muricholic acids decreased in ileal bypass rats. Feeding of the cholesterol diet decreased deoxycholic acid in both normal and ileal bypass rats. The CA/CDCA ratio increased remarkably in ileal bypass rats, and the ratios decreased after being fed the cholesterol diet in both rat groups.

Table 4. Effects of ileal bypass on fecal bile acid composition (%) in rats

	Normal rats		Ileal bypass rats	
	Ordinary diet	Cholesterol diet	Ordinary diet	Cholesterol diet
Lithocholic acid	7.6 ± 0.41	6.5 ± 0.48	11.7 ± 2.11*	11.1 ± 1.94**
Deoxycholic acid	19.5 ± 1.21	13.3 ± 0.50*	56.5 ± 1.32*	43.0 ± 5.07**,***
α-Muricholic acid	4.1 ± 0.35	5.2 ± 1.24	5.4 ± 1.26	6.6 ± 1.40
Hyodeoxycholic acid	6.5 ± 1.59	10.9 ± 4.30	5.9 ± 1.55	9.8 ± 6.70
β-Muricholic acid¶	10.7 ± 3.40	15.6 ± 4.73	3.6 ± 0.82*	4.7 ± 0.61**
ω-Muricholic acid	40.6 ± 8.06	36.3 ± 4.87	9.6 ± 2.54*	19.9 ± 4.86**
Oxo-bile acids	2.3 ± 0.23	ND	5.4 ± 3.22	ND
Others	7.5 ± 3.92	6.7 ± 2.36	1.8 ± 0.45	4.9 ± 2.20
CA/CDCA ratio	0.37 ± 0.112	0.17 ± 0.013*	1.62 ± 0.25*	0.85 ± 0.20**,***

Mean ± SE.
CA/CDCA ratio, ratio of the sum of bile acids derived from cholic acid over the sum of bile acids derived from chenodeoxycholic acid; ND, not detectable.
*Statistically significant compared with normal rats on the ordinary diet ($P < 0.05$).
**Statistically significant compared with normal rats on the cholesterol diet ($P < 0.05$).
***Statistically significant compared with ileal bypass rats on the ordinary diet ($P < 0.05$).
¶This fraction contains a small amount (< 10%) of cholic acid.

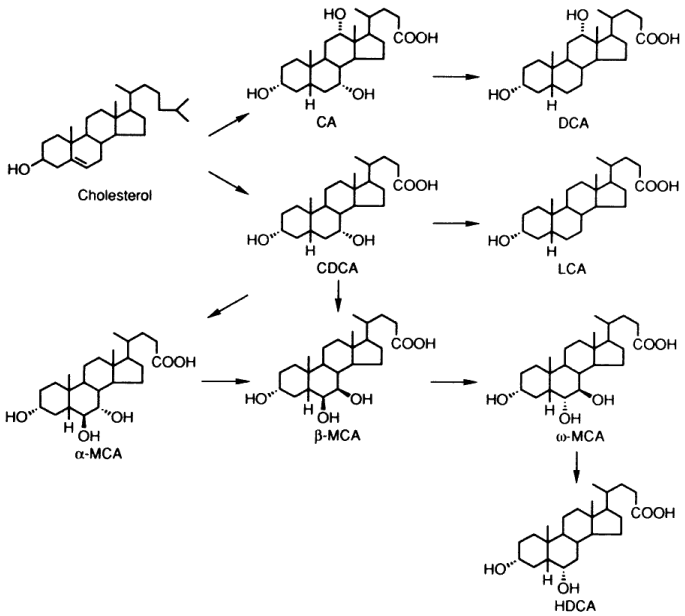

Fig. 1. Metabolic sequences in the primary and secondary bile acid formation in rats.

Discussion

An important step in the enterohepatic circulation of bile acids is the absorption of bile acids from the distal ileum. The major part of bile acids is absorbed there by the active transport mechanism (Dietschy, 1968; Schiff et al., 1972), and the amount comes up to about 60% of the bile acids secreted daily from the liver in rats (Kanamura, 1982).

Since bile acid synthesis in the liver is regulated by the amount of bile acids returning to the liver through the portal vein, the synthesis of bile acids increases by ileal resection (Weis and Dietschy, 1974; Kanamura, 1982) or bypass operation (Kobayashi, 1980) or treatments with bile acid absorption inhibitors (Gustafsson et al., 1978). In a steady state, the daily amount of bile acids excreted into the feces is considered to represent the daily amount of bile acids synthesized in the liver. Therefore, an increase in the synthesis of bile acids is reflected as an increase in fecal bile acid excretion.

The present data demonstrates clearly that the biliary secretion of bile acids decreases and the fecal excretion of bile acids increases in ileal bypass rats. We found that the hourly biliary secretion of bile acids decreased to about 35% that of, and the daily fecal excretion of bile acids increased about 4-fold that of the paired control rats kept on the ordinary diet. These data are consistent with our previous experiments on ileal resection rats (Kanamura, 1982).

On the other hand, cholesterol feeding increases biliary and fecal bile acids in rats (Uchida et al., 1977, 1996). In the present experiments, the increase in biliary bile acid secretion was obscure, but fecal bile acid excretion increased in normal rats after cholesterol feeding. However, no further increase in fecal bile acid excretion was found in ileal bypass rats after cholesterol feeding. This could be due to hepatic bile acid synthesis having attained a maximal level after ileal bypass and being unable to respond to further cholesterol feeding. This supposition, however, is not always consistent with the observations on the bile acid composition and the liver cholesterol level.

Since cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis, is increased by an increment in hepatic cholesterol concentration through a mechanism involving nuclear receptors, especially the liver X receptor (Peet et al., 1998), hepatic bile acid synthesis, and therefore fecal bile acid excretion, increases during cholesterol feeding (Uchida et al., 1977, 1996). On this occasion, the synthesis of chenodeoxycholic acid (actually β-muricholic acid in rats) is more enhanced than that of cholic acid, resulting in a decrease in the CA/CDCA ratio (Uchida et al., 1977, 1996). These changes after cholesterol feeding, an increase in the liver cholesterol level and a decrease in the fecal CA/CDCA ratio, were observed in the present normal rats, and also in the ileal bypass rats. These data suggest that cholesterol feeding affected bile acid synthesis even in ileal bypass rats, while not changing the total amount of bile acids synthesized.

The pool size of bile acids was not detected in the present experiments, but taking account of the decrease in the biliary bile acid secretion in the present series of experiments, and our previous results on the ileum resected rats (Kanamura, 1982; Ogura et al., 1993) or others (Weis and Dietschy, 1974), it will be safe to say that the pool size of bile acids was also decreased in ileal bypass rats. The decrease in the pool size of bile acids, especially that of cholic acid, in ileal bypass rats resulted in a decrease in the serum cholesterol level, an increase in fecal cholesterol excretion, and no increase in the serum cholesterol level after cholesterol feeding.

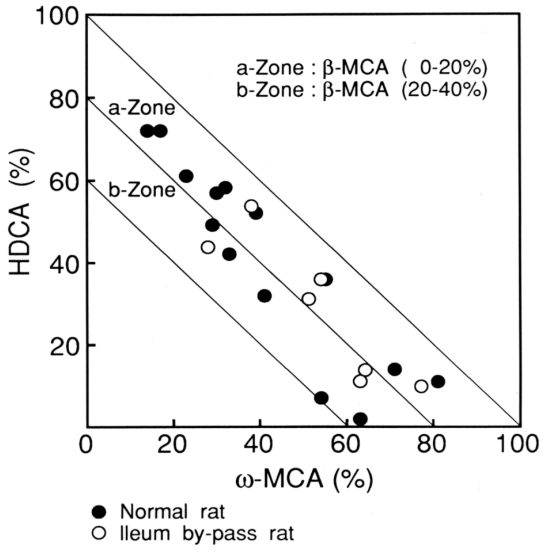

Fig. 2. Relationship between ω-muricholic acid (ω-MCA) and hyodeoxycholic acid (HDCA) formed from β-muricholic acid in rats.

The lithogenic index was low in rats and not affected by cholesterol feeding, but increased slightly in ileal bypass rats. The present data are consistent with the report done by Kulneff-Herlin et al. (1983). The results may support the concept that ileal resection or bypass increases the risk of cholelithiasis (Bickerstaff and Moossa, 1983; Pitt et al., 1984).

The composition ratio of deoxycholic acid increased in ileal bypass rats as shown in the results. Conjugated cholic acid (mostly taurine conjugate in rats) is effectively absorbed from the distal ileum by the active transport mechanism in normal rats (Dietschy, 1968; Schiff et al., 1972). However, a large amount of conjugated cholic acid enters the colon in ileal bypass rats and receives modifications such as deconjugation and 7α-dehydroxylation by intestinal flora (Macdonald et al., 1983; Groh et al., 1993), producing deoxycholic acid. Since a part of the secondary bile acids is also absorbed from the colon, especially the proximal colon (Schiff et al., 1972), the increase in biliary deoxycholic acid is a result of the copious formation of this bile acid in the colon.

The composition ratios of hyodeoxycholic, β-muricholic and ω-muricholic acids in the feces showed relatively large variations. Since β-muricholic acid is considered to be transformed into ω-muricholic acid and then into hyodeoxycholic acid by intestinal flora (Fig. 1), the conversion rates differ from individual to individual according to their intestinal flora. Therefore, the necessary presentation of the ratios of each bile acid in the sum of these three bile acids are calculated and plotted in Fig. 2. An inverse correlation was observed between hyodeoxycholic acid and ω-muricholic acid, suggesting that a copious formation of hyode-

oxycholic acid resulted in a smaller percentage of ω-muricholic acid, and vice versa.

Coprostanol is also formed from cholesterol by intestinal flora (Björkem and Gustafsson, 1971; Ren et al., 1996). In normal rats kept on an ordinary diet which contained about 0.1% or less of cholesterol, the amount of coprostanol in the feces was almost the same as that of cholesterol. After being fed the cholesterol diet, the amount of coprostanol increased about 10-fold, but no significant difference was found between the normal and ileal bypass rats.

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