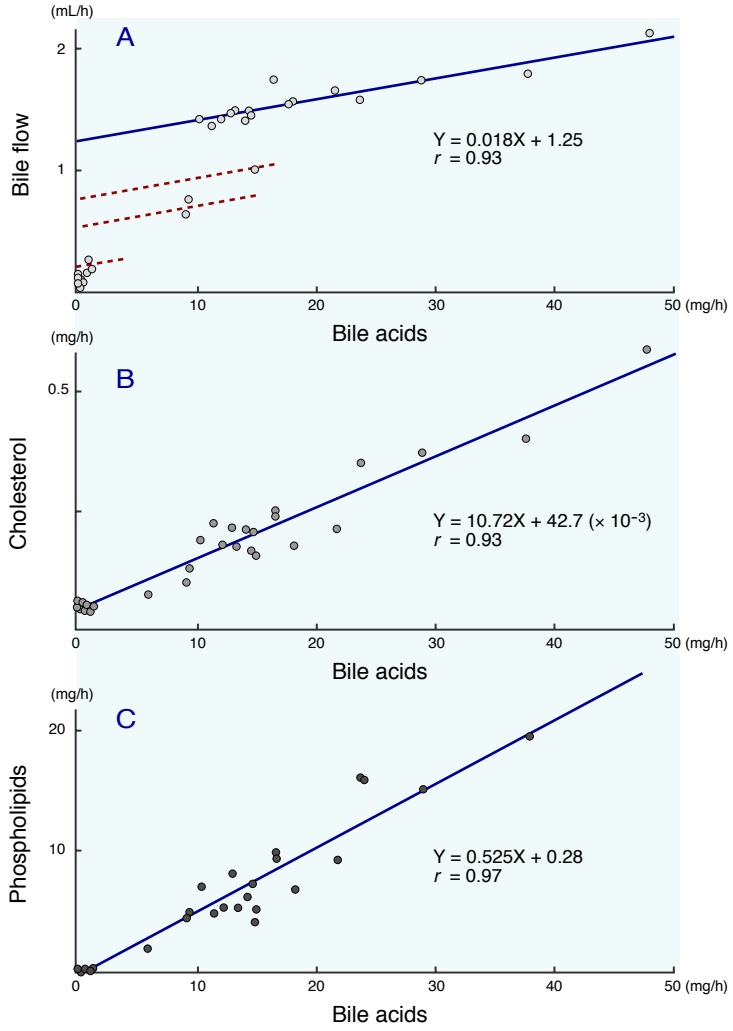

Fig. 1. Correlations between biliary bile acid secretion and bile flow (**A**), biliary bile acid secretion and biliary cholesterol secretion (**B**) and biliary bile acid secretion and biliary phospholipid secretion (**C**) in controls and ANIT treated rats. Broken lines in (**A**) are given under the assumption that the bile acid dependent bile flow is not changed by ANIT (see text).

is assumed that bile acid dependent bile flow is not influenced by ANIT. Based on this assumption, dots representing the bile acid secretion in the region less than 10 mg/h are considered to be on the regression lines shown by broken lines having the same slope with the solid line.

Fukumoto et al. (1980) and Lock et al. (1982) reported that the bile acid independent bile flow was inhibited in ANIT treated rats from a study measuring the biliary clearance of erythritol. This conclusion was consistent with the present data (Fig. 1A). Furthermore, the bile flow in ANIT treated rats markedly increased on day 4, but this increase was due to the bile acid dependent bile flow, because the plots for day 4 were completely matched with the regression line between biliary bile acid secretion and bile flow (Fig. 1A). On the other hand, the cholesterol and phospholipid secretions were linearly related to the bile acid secretion closing the origin (Figs. 1B and C), and the molar ratio of cholesterol and phospholipid in the bile was 1:22.

Table 7. Changes in serum and liver lipid levels after α-naphthylisothiocyanate (ANIT) administration in rats

	Days after ANIT administration					
	0	1	2	4	6	10
Body weight (g)	328 ± 3.5	311 ± 3.3*	302 ± 4.9*	309 ± 4.9*	304 ± 4.2*	309 ± 4.9*
Serum total cholesterol (mg/dL)	67 ± 1.7	147 ± 10.1*	204 ± 20.0*	170 ± 6.9*	84 ± 3.3*	75 ± 3.5
Phospholipids (mg/dL)	134 ± 1.4	305 ± 26.3*	375 ± 46.3*	257 ± 13.8	150 ± 11.3	135 ± 3.7
Triglyceride (mg/dL)	84 ± 5.0	71 ± 3.0*	63 ± 4.6*	63 ± 3.5*	63 ± 4.0*	60 ± 2.2*
C/P ratio	0.50 ± 0.017	0.48 ± 0.014	0.55 ± 0.019	0.66 ± 0.024*	0.56 ± 0.020	0.56 ± 0.014*
Liver weight (g/100g body weight)	3.8 ± 0.10	3.7 ± 0.06	4.1 ± 0.06*	4.6 ± 0.07*	4.2 ± 0.09*	4.0 ± 0.08
Total cholesterol (mg/g)	3.3 ± 0.07	3.5 ± 0.16	3.8 ± 0.09*	3.7 ± 0.08*	3.5 ± 0.07*	3.5 ± 0.03*
Phospholipids (mg/g)	46.9 ± 0.83	49.1 ± 0.67	46.1 ± 1.17	45.3 ± 0.79	45.5 ± 1.13	45.0 ± 0.69
Triglyceride (mg/g)	3.7 ± 0.18	4.7 ± 0.56	2.9 ± 0.28*	4. 3 ± 0.17	3.1 ± 0.16*	3.0 ± 0.16*

Mean ± SE in 5 rats.
C/P, cholesterol/phospholipids.
* Statistically significant compared with day 0 ($P < 0.05$).

This value is not consistent with the cholesterol and phospholipid ratio in the liver membrane of rats (Daum, 1985).

Biliary bile acid excretion is under the regulation of transporters, the bile salt export pump (bsep) (Gerloff et al., 1998) and multidrug resistance associated protein 2 (mrp2) (Paulusma et al., 1996), in the canalicular membrane of the hepatocyte. Bsep is concerned with the excretion of amino acid conjugated bile acids and mrp2 with the excretion of bile acid sulfates and glucuronides. Biliary cholesterol and phospholipid secretions are related to multidrug resistance 2 (mdr2) P-glycoprotein. Phospholipid secretion is completely, and the cholesterol secretion is partially, dependent on *mdr2* gene expression (Oude Elferink et al., 1996).

Cholestasis induced by lipopolysaccharide (Kubitz et al., 1999; Lee et al., 2000), phalloidin (Rost et al., 1999), ethinylestradiol (Lee et al., 2000), or bile duct ligation (Lee et al., 2000; Paulusma et al., 2000) is accompanied by changes in bsep and/or mrp2. ANIT produced cholestasis and decreased biliary cholesterol and phospholipid secretion in parallel with the bile acid secretion (Figs. 1B and C), but ANIT is reported to have no effect on mrp2 (Ogawa et al., 2000). The effect of ANIT on bsep is not yet known.

β-Muricholic acid in the biliary bile aids increased especially on day 4, the day when the biliary bile acid secretion markedly increased, with a concomitant decrease in cholic acid. These changes, however, were normalized on day 6 or later. Maeyama (1977) also reported an increase of β-muricholic acid in ANIT treated rats even after the bile flow was recovered. The increase of β-muricholic acid and a decrease of cholic acid were also found in the serum, urine and feces. In the feces, the increase of β-muricholic acid was found as the increase of ω-muricholic acid formed from β-muricholic acid by the action of intestinal bacteria.

The increase of β-muricholic acid is also reported in bile duct ligated rats (Kinugasa et al., 1981; Takita et al., 1988; Fujio et al., 1989), indicating that cholestasis causes an increase in β-muricholic acid formation. Takita et al. (1988) suggested that an alternative metabolic pathway in bile acid synthesis via 26-hydroxycholesterol, 3β-hydroxy-5-cholenoic acid and lithocholic acid contributes to the increase of β-muricholic acid in bile duct ligated rats. β-Muricholic acid formation is known to increase after the feeding of cholesterol in rats (Uchida et al., 1977), which results in an elevation of the liver cholesterol level. In the case of ANIT treatment (the present experiment) and bile duct ligation (Kinugasa et al., 1981), a minor or no increase in the liver cholesterol level was found, though significant hypercholesterolemia was produced. The mechanism for this increase of β-muricholic acid in cholestasis is not yet known.

The serum bile acid concentration markedly increased after ANIT treatment and the urinary bile acid excretion increased being delayed about 1 day more than the increase of the serum bile acids. The enormous amounts of urinary bile acids on day 2 came, we expect, from the bile acid pool in the enterohepatic circulation.

Fecal bile acid excretion decreased during cholestasis, but increased after day 5 or later exceeding the initial level. This increase is a reflection of the increase of the bile acid synthesis in the liver. Hepatic cholesterol synthesis and cholesterol 7α-hydroxylation activities are reported to increase in bile duct ligated rats (Danielsson, 1973; Cooper and Ockner, 1974; Adler et al., 1977). When one lobe of the liver was experimentally ligated, cholesterol synthesis increased only in the ligated lobe (Cooper and Ockner, 1974; Adler et al., 1977). Therefore, cholestasis is considered to increase hepatic cholesterol synthesis, and probably subsequent bile acid synthesis, in rats.

Fecal sterol excretion decreased on days 1–2 reflecting a decrease in feces dry weight, but markedly increased on days 3–4 and then gradually decreased to the initial level. The composition ratios of coprostanol and cholesterol were almost identical through out the experimental period, suggesting that the intestinal bacteria concerned with the sterol transformation were not changed by ANIT treatment. On the other hand, the secondary bile acid composition was changed after ANIT treatment. ω-Muricholic acid increased and hyodeoxycholic acid decreased after days 5–6 or later. β-Muricholic acid is transformed to ω-muricholic acid and then to hyodeoxycholic acid by the intestinal bacteria. Therefore, the intestinal bacteria concerned with the bile acid metabolism were changed in the ANIT treated rats.

The serum cholesterol and phospholipid levels increased for 4 to 5 days after ANIT treatment. Since enhancement of cholesterol absorption is not the reason, the increase may be a reflection of biliary epithelial cell necrosis (Orsler et al., 1999).

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