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### Bile Acid Composition in the Fistula Bile from Fasted, Cholesterol-Fed or Thyroid Powder-Fed Rats<sup>1)</sup>

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TLC-analysis for bile acid composition was carried out on the bile samples obtained by bile duct-cannulation from the rats fasted for 48 hr and from those kept continuously on a diet supplemented with 1% cholesterol or 2% thyroid powder.

The secretion of total taurine-conjugated bile acids decreased after fasting, only except the secretion of taurodeoxycholic acid which was maintained at normal level. The fasted group, thus, demonstrated an increase in the ratio of trihydroxy to dihydroxy bile acids and a decrease in another ratio of bile acid components, (taurocholic acid + taurodeoxycholic acid)/(taurochenodeoxycholic acid + tauromuricholic acid + taurohyodeoxycholic acid), as compared with the normal group.

Cholesterol-feeding increased slightly the biliary output of total bile acids. However, both of the above ratios for this group were nearly the same to those for the normal group. On the other hand, thyroid powder-feeding caused an increase of taurocholic acid accompanied by a concomitant decrease of taurodihydroxy bile acids within the normal range of total bile acid secretion, resulting in increases of both the ratios over the normal levels.

In all the experimental groups including the normal, the incorporation of intravenously injected taurine-<sup>35</sup>S into biliary taurocholic, tauromuricholic and taurodihydroxy bile acids was approximately proportional to the secreted amounts of these fractions.

Bile acids are the main end products of cholesterol catabolism.<sup>3)</sup> In rats, the two different metabolic pathways are involved, namely, cholesterol-cholic acid and cholesterol-chenodeoxycholic- $\alpha$ - and  $\beta$ -muricholic acid systems.<sup>4)</sup> Cholic, chenodeoxycholic, and muricholic acids are secreted into the bile mostly as taurine-conjugates.<sup>5)</sup> In addition to these primary bile acids, the other constituents of bile acids are also present in the rat bile: deoxycholic and hyodeoxycholic acids are the major bile acids which are formed from cholic and chenodeoxycholic acids, respectively, by the action of intestinal micro-organisms.<sup>6)</sup>

In general, bile acids and their bacterial metabolites are absorbed rapidly from the intestine, transported via the portal vessels to the liver and re-secreted in the bile. It has been proposed that this cyclic process, which is referred as the enterohepatic circulation, may have a regulatory influence upon cholesterol metabolism and bile acid production in the liver.<sup>7)</sup> Bile acid composition in the bile, therefore, is of much importance as an index of this feed-back regulation.

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- 2) Location: a) Takata-3-chome, Toshimaku, Tokyo; b) Yayoicho, Chiba.
- 3) H. Danielsson, *Adv. Lipid Res.*, **1**, 36 (1963).
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In the present study, a thin-layer chromatographic (TLC) analysis was carried out for the taurine-conjugated bile acids in the fistula bile obtained by bile duct-cannulation from normal rats and those fasted and fed excess cholesterol or thyroid powder.

### Experimental

**Animal Experiment**—A commercial pellet (Oriental MF) was used as basal diet. Cholesterol (Kokusan-Kagaku) and thyroid powder (Teikoku-Zohki) were incorporated separately into the basal diet at the rate of 1% and 2%, respectively. Male Wistar rats weighing about 160 g were divided into four groups of 5 animals each. One group was free access to the basal diet and served as the normal control. The second group was fasted by withdrawal of the basal diet at 48 hr prior to bile duct-cannulation. The third group was fed the cholesterol-diet for 4 weeks, and the fourth group received the thyroid powder-diet for one week. The feedings were continued just until the time of cannulation. Bile duct-cannulation was carried out as follows. The abdomen of each animal was incised under ether-anesthesia and the common bile duct was cannulated with a fine polyethylene tube through the duodenal wall where the duct opens. One hour after the cannulation, a trace amount of taurine-<sup>35</sup>S (30  $\mu$ Ci, RCC) was injected intravenously to the animal and bile was collected every one hour for successive 4 hr.

**Measurements of Total Solid Weight and Total Radioactivity**—An aliquot of each bile sample (0.1 ml) was pipetted into a dish of known weight, and dried to a constant weight under infrared lamp. Radioactivity was measured with an end window Geiger-Müller counter.

**Bile Acid Analysis**—Fresh bile samples of 10  $\mu$ l each were applied to a glass plate coated with Silica Gel-H (Merck) and taurine-conjugated bile acids were separated into three fractions by the TLC-technique described previously.<sup>8)</sup> The plate after TLC was sprayed with 50% sulfuric acid and heated at 150° for 20 min. The color density and radioactivity of each fraction were measured using a TLC-radioscanner equipped with a densitometer (Aloka TLC-2B). To obtain the calibration curve for conjugated bile acids, different amounts of taurocholic acid (Sigma) were chromatographed simultaneously with the bile samples. Alternatively, the taurodihydroxy bile acid fraction thus separated was extracted with methanol from another non-color developed plate and subjected to alkaline hydrolysis and petroleum ether-extraction. Individual dihydroxy bile acids were separated by the TLC-system for free bile acids of Mitropoulos and Myant,<sup>4b)</sup> and estimated quantitatively by measuring the color density developed after a spray of 30% phosphomolybdic acid followed by heating at 70° for 20 min.<sup>9)</sup> Standard chenodeoxycholic acid, hyodeoxycholic acid (Mann) and deoxycholic acid (Sigma) were used as reference compounds for calibration. A very small amount of ursodeoxycholic acid was found occasionally in the chromatograms of the extracts but excluded from estimation.

### Result

#### Bile Flow and Solid Secretion

As can be seen in Fig. 1, all the experimental groups including the normal demonstrated gradual decreases in bile flow and solid secretion by the elapsing time of cannulation, keeping biliary total solids at a fairly constant concentration during the bile-draining period.

Fasting decreased the secretion of bile and solids to approximately one half the normal levels ( $p < 0.05$ ). A decrease and an increase in bile flow were observed in the animals fed excess cholesterol and thyroid powder, respectively, despite of the solid secretion of the same order of magnitude in both groups as in the normal. Thus, biliary solid concentration was slightly higher in cholesterol-fed rats ( $p < 0.05$ ) and relatively lower in thyroid powder-fed rats ( $p < 0.05$ ), respectively, than in normal controls.

#### Secretion of Taurine-Conjugated Bile Acids in the Bile

Under each experimental condition, almost of the bile acids secreted in the bile were in the form of conjugates with taurine. The time-course of secretion of taurocholic acid (TC), tauromuricholic acid (TMC) and taurodihydroxy bile acid (TDiBA) fractions in each group is shown in Fig. 2. The average rate of secretion of individual conjugates, TC, TMC, taurochenodeoxycholic acid (TCDC), taurodeoxycholic acid (TDC) and taurohyodeoxycholic acid

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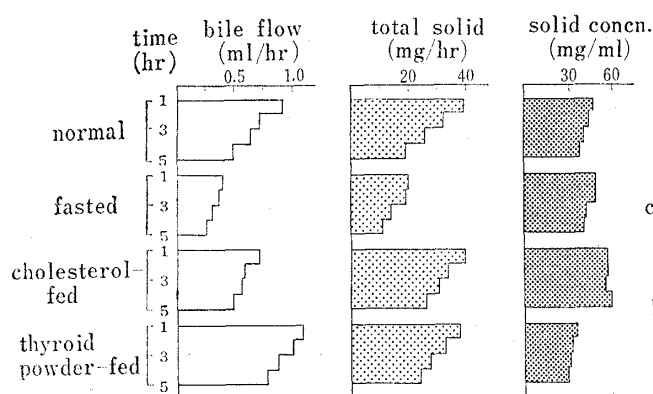


Fig. 1. Bile Flow and Biliary Solid Secretion from 1 hr to 5 hr after Bile Duct-Cannulation

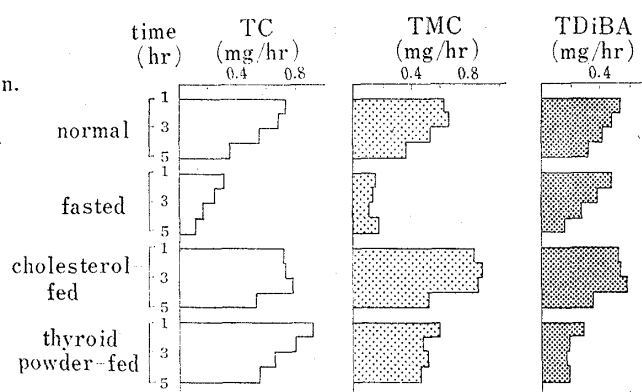


Fig. 2. Biliary Secretion of Taurocholic Acid (TC), Tauromuricholic Acid (TMC) and Taurodihydroxy Bile Acid (TDiBA) Fractions in Bile Duct-Cannulated Rats

TABLE I. Average Rate of Secretion of Individual Taurine-Conjugated Bile Acids (mg/hr)

Group	TriBAs		DiBAs			Total
	TC	TMC	TCDC	TDC	THDC	
Normal	0.59(37.4)	0.55(34.8)	0.18(11.4)	0.10( 6.3)	0.16(10.1)	1.58(100)
Fasted	0.22(33.9)	0.11(16.9)	0.09(13.8)	0.16(24.6)	0.07(10.8)	0.65(100)
Cholesterol-Fed	0.69(35.0)	0.78(39.6)	0.21(10.7)	0.20(10.2)	0.09( 4.6)	1.97(100)
Thyroid Powder-Fed	0.73(50.4)	0.51(35.2)	0.09( 6.2)	0.08( 5.5)	0.04( 2.8)	1.45(100)

( ): %

(THDC), was calculated from the total secreted amounts obtained from one to five hours after the cannulation. The result is summarized in Table I.

In normal controls, total bile acid secretion decreased gradually after the cannulation. However, the ratio of trihydroxy to dihydroxy bile acids (TriBA/DiBA) was kept constant during the bile drainage. Both TriBAs of TC and TMC were secreted into the bile at an approximately equal rate. TDC and THDC, which are the bacterial metabolites of TC and TCDC, respectively, made up about 59% of total TDiBAs. Thus, the ratio between the secreted amounts of bile acids derived from the two metabolic pathways,  $(TC + TDC)/(TCDC + TMC + THDC)$ , was calculated to be about 0.78.

The biliary output of total bile acids decreased markedly in fasted rats, as compared to control ( $p < 0.01$ ). However, the fall of TDiBAs was not so much as those of TC and TMC. This was only due to the TDC secretion sustained at normal level. The fasted group, thus, demonstrated a ratio TriBA/DiBA and an increased ratio  $(TC + TDC)/(TMC + TCDC + THDC)$ .

All the fractions of TC, TMC and TDiBAs tended to be increased to a larger extent in cholesterol-fed rats than in normal controls ( $p < 0.1$ ). However, both of the ratios for the treated group were not different from those for the normal group.

In thyroid powder-fed rats, a slight but not significant increase of TC accompanied by a marked decrease of TDiBAs ( $p < 0.01$ ), was observed within the normal range of total bile acid secretion. The ratios TriBA/DiBA and  $(TC + TDC)/(TMC + TCDC + THDC)$  for this group were about 5.9 and 1.27, respectively, in contrast to 2.6 and 0.78 for the normal.

#### Incorporation of Taurine-<sup>35</sup>S into Taurine-Conjugated Bile Acids

Fig. 3 shows the changes in proportion of the secreted amounts of TC, TMC and TDiBAs, and of the radioactivity incorporated into these fractions after the intravenous injection of taurine-<sup>35</sup>S.

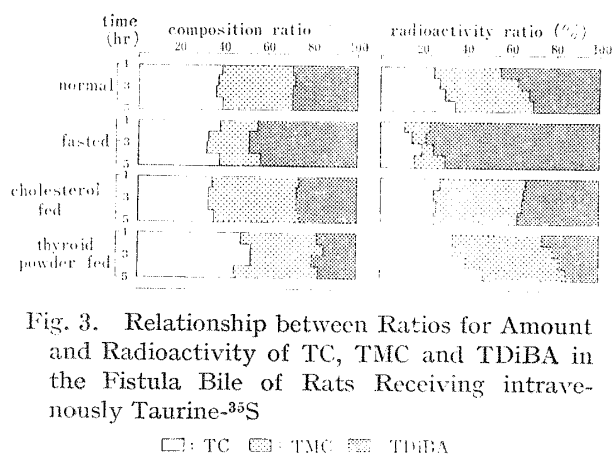


Fig. 3. Relationship between Ratios for Amount and Radioactivity of TC, TMC and TDiBA in the Fistula Bile of Rats Receiving intravenously Taurine-<sup>35</sup>S

□ TC ▨ TMC ■ TDiBA

In the bile of all the animals including normal controls, the TC, TMC and TDiBAs secreted were labeled with taurine-<sup>35</sup>S approximately in proportion to their amounts. Nevertheless, the radioactivity ratio was relatively high in TDiBAs and in contrast low in TC, as compared with the amount ratio. This tendency was noted clearly in all the bile samples from the fasted rats, and in the early collected samples from the normal and thyroid powdered rats.

### Discussion

The TLC-method employed in the present study is simple and adequate to routine work for group-separation of taurine-conjugated bile acids from the rat bile. To separate TMC from TC without hydrolysis is necessary not only because these TriBAs are derived from the different pathways of cholesterol catabolism,<sup>4)</sup> but also because  $\alpha$ - and  $\beta$ -muricholic acids are relatively labile to alkaline hydrolysis and solvent extraction (unpublished data). The value for TMC presented in this paper is somewhat larger than that which might be expected from the original paper of Matschiner, *et al.*<sup>10)</sup>

The normal ratio TriBA/DiBA for the rat bile obtained in this work, however, is quite similar to those for the rat liver homogenate, portal plasma and small intestinal contents determined by the previous authors<sup>11)</sup> using gaschromatographic techniques. The similarity found between our and the others' data suggest that total bile acids may circulate enterohepatically keeping a constant ratio TriBA/DiBA.

This work demonstrates that bacterial bile acid metabolites constitute more than half the TDiBAs fraction secreted early after cannulation. The presence of deoxycholic and hyodeoxycholic acids in the fistula bile of rats has been reported.<sup>6b,12)</sup> In the rat liver, deoxycholic acid can be converted again to cholic acid by the action of a  $7\alpha$ -hydroxylating system,<sup>4b,13)</sup> while hyodeoxycholic acid is not further metabolized.<sup>6b,14)</sup> Maintenance of the normal level of TDC secretion in the fasted rats showing marked decreases of the other bile acid secretion, as observed in this work, may be due to a decreased activity of the liver enzyme (s) for  $7\alpha$ -hydroxylation. In addition, the conspicuous incorporation of exogenous taurine-<sup>35</sup>S into TDiBAs observed in the fasted rats suggest a possibility that unconjugated deoxycholic acid may be absorbed to a larger extent in fasting state than in feeding state. It has been described that the type of diet fed may have a pronounced influence upon the formation of bacterial bile acid metabolites in the intestine and the proportion of the conjugates and unconjugates to be absorbed.<sup>11b)</sup>

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Several authors<sup>12,15)</sup> have reported that feeding of excess cholesterol or administration of thyroid powder to rats causes a decrease of cholic acid production accompanied by a concomitant increase of chenodeoxycholic acid production. Such an observation, however, is not obtained in the present study. As mentioned in the results of this work, the ratio  $(TC+TDC)/(TCDC+TMC+THDC)$  for the bile may indicate a relative contribution of the two pathways of bile acid production. This ratio is nearly the same between the cholesterol-fed and the normal groups. On the other hand, the rats fed continuously thyroid powder, as well as those fasted for 48 hr, show an increased ratio which suggests a relative stimulation of cholic acid production. It is interesting that these two groups are equally in an excess catabolic state. The present study on bile acid composition in the bile of thyroid powder-fed rats would rather support the finding of Failey Jr., *et al*<sup>16)</sup> that a transitory increase of cholic to chenodeoxycholic acid ratio was observed in the human bile obtained by duodenal intubation 2 hr after administration of L-triiodothyronine.

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