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Effect of Biliary Drainage on Bile Acid Conjugation with Taurine in Vitamin B_6-Deficient Rats[1]

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Feeding of a synthetic, vitamin B_6-deficient diet for 7 weeks caused a marked increase in the ratio of glycine- to taurine-conjugated bile acids in the bile of rats. The highly elevated ratio, however, was reduced spontaneously by the elapsing time of biliary drainage, and the decrease in the ratio was accelerated by a subcutaneous administration of 2 g/kg of taurine.

An intravenous injection of 5 mg/kg of sodium deoxycholate caused a temporary increase in the biliary secretion of taurodeoxycholic acid in both groups of normal and vitamin B_6-deficient animals, and taurine loading prior to the deoxycholate-injection further augmented the increased secretion of this taurine-conjugated bile acid fraction. The glycodeoxycholic acid secretion in vitamin B_6-deficient rats was not increased significantly after the deoxycholate-injection.

Bile acids are present in the mammalian bile as the conjugates with glycine and/or taurine. The ratio of glycine to taurine conjugates (G/T) varies from species to species. The G/T for the human bile is about 3.0. In contrast, the ratio is very low for the rat bile containing more than 95% of total bile acids in the form of conjugation with taurine.[3]

Bergeret and Chatagner[4] reported that vitamin B_6 deficiency in rats caused a marked increase in glycine conjugate secretion with a counterbalanced decrease in taurine conjugate secretion resulting in a marked elevation of G/T.

The present study suggests that the increased G/T in vitamin B_6-deficient rats may have a close relation to the function of the enterohepatic circulation.

Experimental

Male Wistar rats 4 weeks old were kept on a synthetic diet free of vitamin B_6 for 7 weeks.[5] On the other hand, those which were pair-fed the same diet supplemented sufficiently with the vitamin for the identical period served as normal controls. The typical signs of vitamin B_6 deficiency developed in the mucous membrane of eyes and nose, the skin of limbs and tail, and the hair of the body of all animals fed the test diet. At the time of experiments, the common bile duct of all rats was cannulated with a fine polyethylene tube in previously mentioned manner[1] and bile was collected every one hr for successive 6 hr. These aminals were divided into three groups. One group was the non-treated control. The second group received intravenously sodium deoxycholate (Sigma) in the dose of 5 mg/kg 2 hr after the cannulation and the third was treated subcutaneously with taurine (J.P.) at 2 g/kg one hr prior to the deoxycholate-injection.

The thin-layer chromatographic (TLC) and colorimetric methods for bile acid analysis employed in this experiment were the same to those described previously.[1,6]

1) This work was presented partly at the 93rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1973.
2) Location: *Takata-3-chome, Toshimaku, Tokyo.*
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Result

As can be seen in Table I, the average body weight, and bile volume of vitamin B_6-deficient rats were significantly smaller than those of normal controls ($p<0.05$). The biliary output of total bile acids was relatively lower in vitamin B_6-deficient rats than in normal controls ($p<0.1$), but the amount secreted from 1 g of liver was not almost different between both groups only except of a marked increase in the ratio G/T for the deficient group.

In vitamin B_6 deficiency the rate of taurine conjugate secretion decreased largely from 2.10 mg/hr of the normal level to 0.63 mg/hr, while in turn that of glycine conjugate secretion increased up to 0.83 mg/hr from its normal rate which was estimated TLC–densitometrically to be less than 0.10 mg/hr.

As shown in Fig. 1, the increased G/T observed in the first one hr-bile of vitamin B_6-deficient rats, however, was reduced rapidly in the successive periods of biliary drainage, and

Table I. Effect of Vitamin B_6 Deficiency on the Bile Acid Composition

Group	No. of rats	Body weight (g)	Liver weight (g)	Bile flow (ml/hr)	Total B.A. (mg/hr)	G/T	Individual bile acids (mg/hr)					
							Taurine Conj.			Glycine Conj.		
							TC	TMC	TDiBA	GC	GMC	GDiBA
Normal	6	257 ±24[a]	9.37 ±1.65	0.65 ±0.05	2.10 ±0.07	<0.05	0.90 ±0.04	0.71 ±0.03	0.49 ±0.05	<0.10[b]		
Vitamin B_6-deficient	6	161[c] ±9	6.24 ±0.46	0.30[d] ±0.03	1.46 ±0.16	1.32	0.22 ±0.02	0.14 ±0.02	0.27 ±0.02	0.34 ±0.03	0.27 ±0.05	0.22 ±0.05

a) mean ± S.E.
b) The total amounts were too small to be determined exactly by the densitometric method.
c) $p<0.05$
d) $p<0.01$: a significant decrease
TC: taurocholic acid, TMC: tauromuricholic acid, TDiBA: taurodihydroxy bile acids,
GC: glycocholic acid, GMC: glycomuricholic acid, GDiBA: glycodihydroxy bile acids

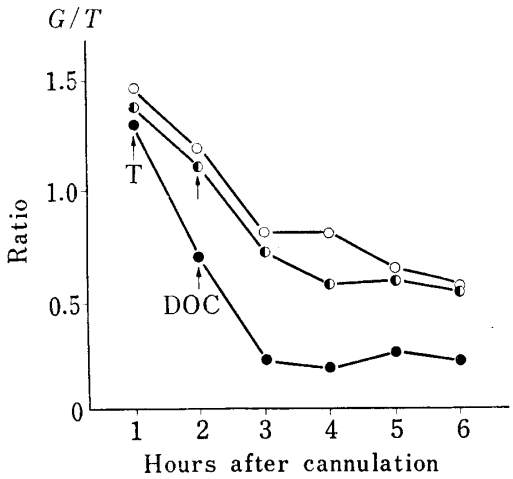

Fig. 1. Decrease in the Ratio of Glycine (G)-to Taurine(T)-conjugated Bile Acids in the Fistula Bile of Vitamin B_6-Deficient Rats

Arrows show the time of administration of sodium deoxycholate (DOC, 5 mg/kg *i.v.*) and taurine (T, 2 g/kg *s.c.*).
—○—: untreated
—◑—: DOC
—●—: DOC+T

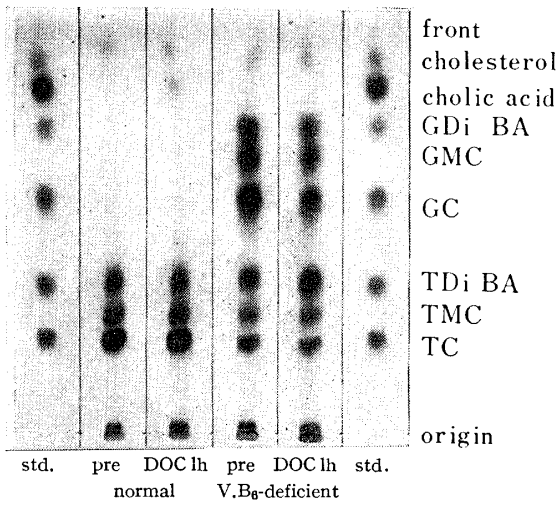

Fig. 2. TLC–Chromatograms of the Bile Samples from Normal and Vitamin B_6-Deficient Rats Receiving Sodium Deoxycholate

pre: before the intravenous injection of sodium deoxycholate, DOC 1 h: 1 hr after the injection, std:reference compounds

such a fall of G/T was further accelerated by the subcutaneous injection of 2 g/kg of taurine. The following injection of sodium deoxycholate at 5 mg/kg had no significant effect on the rate of the decrease in G/T.

Fig. 2 shows the chromatograms of the bile samples obtained before and one hr after the deoxycholate-injection. In the bile from vitamin B_6-deficient rats, bile acids appeared almost in the form of conjugation irrespective of the deoxycholate-injection. On the other hand, the presence of a small amount of the free bile acid chromatographically identical with cholic acid was observed in the normal bile obtained after the deoxycholate-injection.

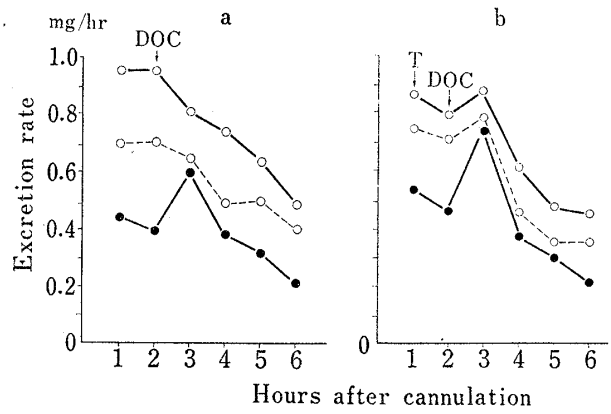

Fig. 3. Biliary Secretion of three Taurine-conjugated Bile Acid Fractions after the Intravenous Injection of Sodium Deoxycholate(a) and the Effect of Taurine Loading before the Injection (b) in Normal Rats

Arrows show the time of administration of deoxycholate (DOC)and taurine (T).
○—○: TC
○---○: TMC
●—●: TDiBA

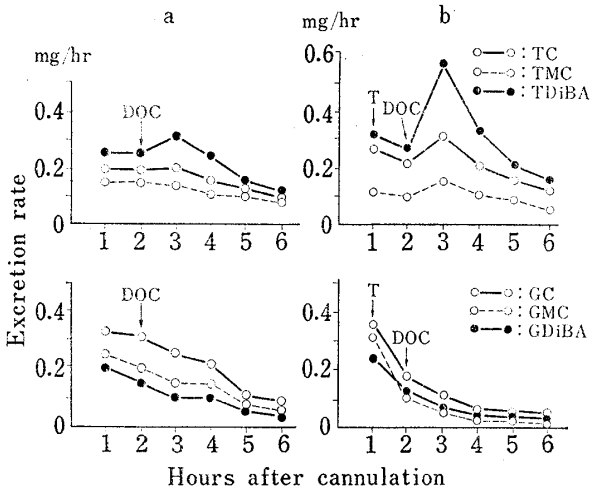

Fig. 4. Biliary Secretion of Bile Acid Fractions after the Intravenous Injection of Sodium Deoxycholate (a) and the Effect of Taurine Loading before the Injection (b) in Vitamin B_6-Deficient Rats

Arrows show the time of administration of deoxycholate (DOC) and taurine (T).

TABLE II. Average Rate of Secretion of Individual Dihydroxy Bile Acids

Group		Time[a] (hr)	Taurine conjugates (mg/hr)				Glycine conjugates (mg/hr)			
			CDC	HDC	DC	Total	CDC	HDC	DC	Total
Normal	I[b]	1	0.14	0.14	0.16	0.44				
		2	0.14	0.15	0.12	0.41				
		3	0.11	0.09	0.38[c]	0.58[d]				
		6	0.07	0.07	0.08	0.22				
	II[e]	1	0.21	0.21	0.19	0.61				
		2	0.19	0.13	0.13	0.45				
		3	0.19	0.12	0.43[c]	0.74[d]				
		6	0.06	0.05	0.11	0.22				
Vitamin B_6-deficient	I[b]	1	0.08	0.10	0.06	0.24	0.09	0.09	0.03	0.21
		2	0.11	0.09	0.05	0.25	0.07	0.05	0.04	0.16
		3	0.04	0.02	0.24[c]	0.30	0.04	0.04	0.03	0.11
		6	0.05	0.01	0.05	0.11	0.01	0.02	0.01	0.04
	II[e]	1	0.08	0.13	0.08	0.29	0.08	0.08	0.07	0.23
		2	0.05	0.15	0.05	0.25	0.04	0.05	0.04	0.13
		3	0.08	0.04	0.43[c]	0.55[d]	0.03	0.02	0.03	0.08
		6	0.04	0.02	0.06	0.12	0.003	0.01	0.007	0.02

a) after bile duct-cannulation
b) given intravenously sodium deoxycholate at the end of 2 hr after cannulation
c) $p<0.01$
d) $p<0.05$; significant increase from the preceding value
e) given subcutaneously taurine 1 hr before the deoxycholate-injection
CDC: chenodeoxycholic acid, HDC: hyodeoxycholic acid, DC: deoxycholic acid

As can be seen in Fig. 3, the intravenous injection of sodium deoxycholate into normal rats resulted in a temporary increase in the biliary secretion of dihydroxy bile acid fraction, and the taurine-loading before the injection produced further increase of this fraction accompanied with a very slight rise of both trihydroxy bile acid fractions in the bile.

As indicated in Fig. 4, similar change also occurred in the pattern of biliary secretion of taurine-conjugated bile acids in the vitamin B_6-deficient rats receiving sodium deoxycholate alone or in combination with taurine. However, no increase was observed in the secretion of glycine-conjugated bile acids in these animals.

Table II summarizes the result of analysis for dihydroxy bile acids on the bile samples from normal and vitamin B_6-deficient rats of both treated groups. In every case, only taurodeoxycholic acid was increased temporarily after the deoxycholate-injection. The relative increase was the highest in the vitamin B_6-deficient rats pretreated with taurine. Both chenodeoxycholic and hyodeoxycholic acids had no relation to the increased secretion of taurine-conjugated dihydroxy bile acids after the deoxycholate-injection.

Discussion

An extremely increased G/T is found clinically in patients with ileal disorder, having an abnormality in the mode of intestinal absorption of bile acids.[7] Usually, the bile acids secreted into the bile are absorbed from the intestine and return to the liver *via* the portal vein. This cyclic process, which is referred to the enterohepatic circulation, takes much important roles in the feed-back regulation of cholesterol-bile acid metabolism in the liver.[3] Conjugation mechanism may be under the influence of this process.

In vitamin B_6-deficient rats, the decrease in taurine-conjugated bile acid secretion accompanied with an increase in glycine-conjugated bile acid secretion occurs within the normal range of total bile acid secretion per 1 g of liver. However, vitamin B_6 deficiency does not cause a decrease in the taurine content of rat liver, brain, spleen or muscle.[8]

The present study shows that the increased G/T in vitamin B_6-deficient rats can be restored spontaneously by the elapsing time of biliary drainage although the fall of the G/T is further accelerated by the administration of taurine, and in addition demonstrates that intravenously administered sodium deoxycholate can be conjugated predominantly with taurine not only in normal rats, but also in vitamin B_6-deficient rats. Recently, an interesting report of the *in vitro* study has been presented that the capacity of liver homogenates to conjugate cholic acid-^{14}C to taurine, as well as that to glycine, was approximately three times greater in vitamin B_6-deficient rats than in normal controls.[9]

These observations lead to the idea that the function of the enterohepatic circulation may be contributory to establishing the increased G/T in vitamin B_6-deficient rats. Namely, the relative decrease in taurine-conjugated bile acid secretion in these animals could be induced by an increase in the quantity of glycine-conjugated bile acids which circulate enterohepatically.

The enhancing effect of taurine-pretreatment on the biliary secretion of administered deoxycholate is another problem to be explained. Exogenous taurine, contrary to exogenous glycine, can be utilized readily for conjugation with bile acids in the rat, rabbit, dog and human.[10] It is reported that a transitory depletion of liver taurine can be produced in dogs

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by continuous infusion of cholic acid into the portal vein and restored by the administration of taurine.[10c] In the same sense, taurine loading before the injection of sodium deoxycholate seems much profitable to the conjugation of the free bile acid to be removed.

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