19. Studies on Bilirubin Metabolism. IV

Deficient Excretion of the Bilirubin Conjugate "C" into the Bile of Congenital Non-hemolytic Hyperbilirubinemia Including Dubin-Johnson, Rotor, and Gilbert's Syndrome^{†)}

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In the previous communication of this series,¹⁾ it was demonstrated that the relative abundance of bilirubin-IX conjugates excreted into normal adult human hepatic bile is approximately diglucuronide, 3%; mono-glucuronide, 35%; and the conjugate "C", newly discovered conjugate, 60%, and biological significance of the major bilirubin conjugate "C" was also discussed.

In the present report, deficient excretion of the bilirubin conjugate "C" in the bile from patients affected by congenital non-hemolytic hyperbilirubinemia is demonstrated by means of high-performance liquid chromatography (HPLC).

Materials and methods. Bile and serum from patients with congenital non-hemolytic hyperbilirubinemia including Dubin-Johnson, Gilbert's, and Rotor syndrome were lyophilized as soon as possible and stored at -80° .

High-performance liquid chromatography (HPLC) has been carried out by the use of Waters ALC/GPC-244 apparatus. Treatment of biological specimens prior to analysis and conditions of the chromatography will be presented elsewhere.²⁾

Results and discussion. As shown in Figs. 1–3 and Table I, there is a significant difference in the ratio of di-glucuronide to conjugate

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Table I. Composition of bilirubin conjugates and free bilirubin isomers in bile and serum from patients with congenital non-hemolytic hyperbilirubinemia

Patient	Sex	Specimen	% Composition				
			di-glu- curonide	mono-glu- curonide	“C”	free IX α	free IX β
Dubin-Johnson syndrome							
F.M.	female	bile	44.3	40.5	0.0	12.0	3.1
K.M.	male	bile	57.8	36.5	0.0	0.7	5.1
		serum	10.1	15.3	0.0	73.4	1.2
S.H.	male	bile	41.3	48.8	3.4	4.8	1.7
Y.M.	male	bile	50.6	40.4	0.0	5.0	4.0
		serum	13.1	12.6	1.2	72.5	0.0
Gilbert's syndrome							
S.I.	male	bile	55.1	40.1	0.0	1.0	3.7
U.S.	male	bile	50.3	45.0	0.6	2.0	2.1
		serum	0.0	0.0	0.0	100.0	0.0
Rotor syndrome							
Y.O.	male	bile	55.6	41.1	0.0	0.3	3.1
		serum	9.4	27.5	1.1	62.0	1.2
(Healthy male)							
I.I.		hepatic bile	2.0	35.0	55.0	5.0	3.0
		serum	0.0	0.0	0.0	100.0	0.0
K.S.		doudenal bile	2.9	32.0	57.0	6.6	1.5

"C" between normal and congenital non-hemolytic hyperbilirubinemic bile.

In four patients, 3 males and 1 female, affected by Dubin-Johnson syndrome, no conjugate "C" is detected in duodenal bile by HPLC excepting in the case of S.H. (3.4%) (Table I). Presenting striking contrast to the above, di-glucuronide, which is less than 3% in normal bile, was increased to 44.3–57.8% in these three cases (50.9% in average).

The same is true in the cases of Gilbert's or Rotor syndrome, though the number of samples was small at the present time.

Isomeric composition of free bilirubins obtained after alkaline hydrolysis of these bile specimens shows no difference with normal bile; IX α isomer, 96–97% ; IX β isomer 3–4%.

The present data show that common features in these congenital non-hemolytic hyperbilirubinemia are deficient excretion of bilirubin conjugate "C" into bile due to either impaired conjugate "C"-forming system or disturbances in conjugate "C"-transporting system. Under these circumstances, bilirubin would be conjugated as di-glucuronide and mono-glucuronide predominantly, and the glucuronides thus formed are considered less effectively excreted into the bile. Deficiency in conjugate "C" excretion does not explain the differences in

the clinical symptoms among three syndromes, and the exact mechanism underlying in these processes are currently under study.

Fig. 1. Separation of bilirubin conjugates and free bilirubin isomers from the bile of the patient affected by Dubin-Johnson syndrome by HPLC. Elution pattern was monitored at 436 nm and 365 nm. Note the deficient excretion of bilirubin conjugate "C" when compared with those of control subject.

Fig. 2. Separation of bilirubin conjugates and free bilirubin isomers from the bile of the patient affected by Gilbert's syndrome by means of HPLC.

Fig. 3. Separation of bilirubin conjugates and free bilirubin isomers from the bile of the patient affected by Rotor syndrome by means of HPLC.

References

- 1) Yamaguchi, T., Yamaguchi, N., Nakajima, H., Komoda, Y., and Ishikawa, M.: Proc. Japan Acad., 55B, 89-93 (1979).
- 2) —: (In preparation).