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A New Solvent System for Thin-Layer Chromatographical Separation of Taurine-conjugated Trihydroxy Bile Acids in Rat Bile

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In the rat bile, cholic acid is the major trihydroxy bile acid.²⁾ The presence of small amounts of additional trihydroxy bile acids, α - and β -muricholic acids, has been reported.³⁾ Although these bile acids are the end products of cholesterol catabolism, α - and β -muricholic acids are formed by the lithocholic acid-chenodeoxycholic acid pathway which differs from that cholic acid is produced.⁴⁾

Cholic acid and muricholic acids exist in the rat bile as the conjugates mainly with taurine⁵⁾ and can be separated only after deconjugation to each one by chromatographical procedures.^{4a,6)} However, all the methods waste the time for conversion of conjugated bile acids to unconjugated ones and often result in variable loss of components.

In the preceding paper,⁷⁾ the authors studied the incorporation of administered taurine-³⁵S into biliary tri- and dihydroxy bile acid fractions by thin-layer chromatography (TLC) using the Kazuno's solvent system,⁸⁾ and observed discrepancy between the densitogram and the radioactinogram of the taurocholic acid fraction, indicating the contamination of other taurine-conjugated trihydroxy bile acid.

The present paper deals with a TLC-technique using a new solvent system for good separation of the other conjugates from taurocholic acid in the fresh rat bile.

Material and Method

Cholic, deoxycholic, taurocholic and glycocholic acids were obtained from Sigma Chem. Co., and chenodeoxycholic, hyodeoxycholic and hyocholic acids from Mann Research Lab. These compounds were purified before use by standard TLC-techniques. All the solvents and reagents used in the experimental were of analytical grade.

The rat bile was obtained by cannulation of the common bile duct and the bile samples of other species were collected by aspiration of the gall bladder.

- 1) Location: a) Takata-3-chome, Toshimaku, Tokyo; b) Yayoi-cho, Chiba.
- 2) The following trivial names for tri- and dihydroxy-5 β -cholan-24-oic acids are used (position of hydroxyl group in molecule is in parenthesis): cholic acid (3 α , 7 α , 12 α); hyocholic acid (3 α , 6 α , 7 α); α -muricholic acid (3 α , 6 β , 7 α); β -muricholic acid (3 α , 6 β , 7 β); chenodeoxycholic acid (3 α , 7 α); deoxycholic acid (3 α , 12 α); hyodeoxycholic acid (3 α , 6 α).
- 3) J.T. Matschiner, T.A. Mahowald, W.H. Elliott, E.A. Doisy Jr., S.L. Hsia, and E.A. Doisy, *J. Biol. Chem.*, **225**, 771 (1957).
- 4) a) G.D. Charayil, S.L. Hsia, J.T. Matschiner, E.A. Doisy Jr., W.H. Elliott, S.A. Thayer, and E.A. Doisy, *J. Biol. Chem.*, **238**, 1973 (1963); b) K.A. Mitropoulos and N.B. Myant, *Biochem. J.*, **103**, 472 (1967).
- 5) G.A.D. Haslewood, "Bile Salts," 1st ed. Methuen Co., Ltd., London, 1967, pp. 82-106.
- 6) a) P. Eneroth, *J. Lipid Res.*, **4**, 11 (1963); b) T. Cronholm and J. Sjövall, *Eur. J. Biochem.*, **2**, 375 (1967); c) C.M. Siegfried and W.H. Elliott, *J. Lipid Res.*, **9**, 394 (1968).
- 7) A. Minato, S. Hirose, T. Ogiso, K. Uda, Y. Takigawa, and E. Fujihira, *Chem. Pharm. Bull. (Tokyo)*, **17**, 1498 (1969).
- 8) T. Kazuno, *Taisha*, **2**, 869 (1965).

Silica gel H (Merck) was coated 0.25 mm thick on a glass plate and activated at 110–120° for 2 hr. The bile sample (10–20 μ l) was applied as a band and developed ascendingly in the solvent mixture of the following composition; isoamylalcohol-acetone-acetic acid-water (63:26:6:5 by volume). When the solvent front travelled approximately 15 cm from the starting line, the plate was taken out, dried at 120° for 1 hr, allowed to cool to room temperature in a desiccated chamber, and subjected to the second development of TLC using the same solvent system. Finally the plate was dried at 120° for 40 min, sprayed with a 50% sulfuric acid and heated at 120° for 20 min.

Result and Discussion

Figure 1 shows the typical TLC-chromatogram of the rat bile, comparing with those of the bile samples from other species. Reference taurocholic and glycocholic acids gave Spots A and D, respectively. Spot A was dominantly present in rat, mouse, dog and hamster bile, but apparently absent in rabbit and pig bile. It has been known that rat, mouse and dog bile contain exclusively taurine-conjugated bile acids, whereas rabbit and pig bile contain nearly all glycine-conjugates.⁵⁾

The radioactive bile was obtained from the bile duct-cannulated rats receiving taurine-³⁵S and submitted to TLC in the same manner. Figure 2 shows the densitogram and the radioactinogram scanned with a TLC-chromatogram scanner, Aloka Model TLC-2B.

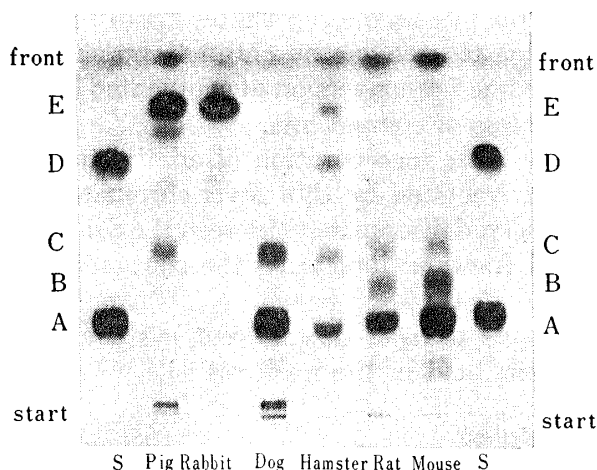


Fig. 1. TLC-Chromatogram of Bile from Six Species Animals

The solvent system was isoamylalcohol-acetone-acetic acid-water (63:26:6:5 by volume). For other details of the TLC-procedure, see the text.

S: standard compounds, taurocholic (A) and glycocholic (D)

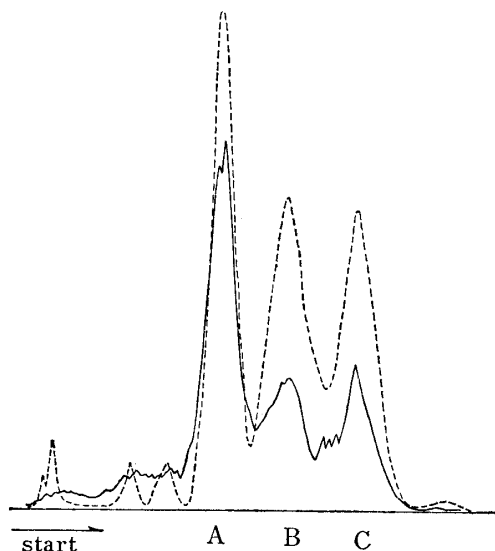


Fig. 2. Densitogram (-----) and Radioactinogram (—) of Bile from Rats Administered Taurine-³⁵S

The radioactivity was located exclusively on the three major spots corresponding to Spots A, B, and C. Individual radioactive portions were scraped off from the plate, extracted with methanol and chromatographed by using standard TLC-solvent systems for conjugated bile acid analysis.^{8,9)} A trace amount of the radioactivity under Spot A was corresponding to free taurine-³⁵S. Spot A was the same in TLC-behavior as taurocholic acid. Good separation was obtained invariably either between A and C, or B and C, but not between A and B.

The bile acid fraction was isolated from each methanol extract by alkaline-hydrolysis and petroleum ether-extraction,⁵⁾ and analysed for absorption spectrum in 65 %H₂SO₄¹⁰⁾

9) a) A.F. Hofmann, *J. Lipid Res.*, **3**, 127 (1962); b) R. Shioda, P.D.S. Wood, and L.W. Kinsell, *J. Lipid Res.*, **10**, 546 (1969).

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and relative TLC-mobility in appropriate solvent systems. Spot A was determined to contain cholic acid only, while Spot C was the mixture of two dihydroxy bile acids, chenodeoxycholic and hyodeoxycholic acids. These bile acids are the normal constituents of the rat bile.⁵⁾

Spot B after hydrolysis was separated regularly into two fractions, each having a TLC-mobility different from those of cholic and hyocholic acids, when submitted to TLC using either of System 7 and 8 of Eneroth,^{6a)} or System A of Siegfried, *et al.*^{6c)} Comparative study of sulfuric acid absorption spectrum indicated that either of the two fractions was clearly distinct from cholic acid and similar to hyocholic acid. However, hyocholic acid is the species-specific bile acid present only in the pig bile.⁵⁾ Hsia, *et al.*¹¹⁾ have described that β -muricholic acid shows a similar absorption spectrum to that of hyocholic acid. A major one of the two fractions was identical with β -muricholic acid which was isolated from the urine of surgically jaundiced rats receiving hyodeoxycholic acid according to the method of Matschiner, *et al.*¹²⁾ The minor one was assumed to be α -muricholic acid from the TLC-behavior and the absorption spectrum, although the reference compound was unavailable.

The TLC-procedure described in the present paper is simple, rapid and accurate for separation of the taurine-conjugated trihydroxy bile acids in the rat bile and useful in studying the cholesterol and bile acid metabolism in rats.

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- 11) S.L. Hsia, J.T. Matschiner, T.A. Mahowald, W.H. Elliott, E.A. Doisy Jr., S.A. Thayer, and E.A. Doisy, *J. Biol. Chem.*, **225**, 811 (1957).
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On the Electronic Interaction of Cobalt Tetraphenylporphine with Tetracyanoethylene as Investigated by ESR

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We have previously²⁾ investigated the reaction of synthetic cobalt tetraphenylporphine (Co-TPP) with molecular oxygen by electron spin resonance (ESR). As a continuation and extension of the work, the electronic interaction of Co-TPP with tetracyanoethylene (TCNE) was now investigated with special interest in π -bonding nature of TCNE. The latter is well-known to be a strong π -acid forming a number of adducts with metal complexes.³⁻⁵⁾

- 1) Location: Bunkyo-ku, Hongo 7-3-1, Tokyo.
- 2) K. Yamamoto and T. Kwan, *J. Catalysis*, **18**, 354 (1970).
- 3) O.W. Webster, W. Mahler, and R.E. Benson, *J. Am. Chem. Soc.*, **84**, 3678 (1962).
- 4) W.H. Baddley, *J. Am. Chem. Soc.*, **88**, 4545 (1966).
- 5) M.F. Rettig and R.M. Wing, *Inorg. Chem.*, **8**, 2685 (1969), and references therein.