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## Note

# Thin-layer chromatography of conjugated bile acids

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Thin-layer chromatography (TLC) has proved to be very useful for the rapid qualitative analysis of free bile acids and solvent systems have been worked out for the separation of most of the bile acids of biochemical interest[1–10]. Little work has, however, been done for the separation of the individual glycine and taurine conjugated bile acids[1–3,8–11], while a group separation of the glycine and the taurine conjugates has recently been reported[12]. In the present paper a solvent system is described which separates the glycine and the taurine conjugates of lithocholic, chenodeoxycholic, deoxycholic and cholic acids from one another. The different glyco- and tauro-conjugates present in human and guinea pig biles have been separated using this system. In addition, sharp differences are demonstrated in the relative mobilities of the various glycine *vs.* taurine conjugated bile acids on changing from an acidic to a basic solvent system.

## EXPERIMENTAL

Taurine and glycine conjugated lithocholic, chenodeoxycholic, deoxycholic and cholic acids (Calbiochem, Los Angeles, Calif., U.S.A.) were dissolved in methanol and 2–3 $\mu$g were applied to 0.25 mm thick silica gel G plates (Brinkmann, Westbury, N.Y., U.S.A.). The plate was developed in the appropriate solvent system and the solvent was allowed to rise 16–18 cm from the starting line, followed by drying at 110°. When necessary, the plate was developed a second time in the same solvent system. The plate was then sprayed with 20% sulfuric acid and phosphomolybdic acid (3.5% in isopropanol; EM Labs, Westbury, N.Y., U.S.A.) and heated at 110° for 2 min. The spots, thus obtained, generally had a maximum diameter of 1 cm.

## RESULTS AND DISCUSSION

Table I illustrates the mobilities of the various conjugated bile acids relative to that of taurocholic acid obtained in a number of acidic and basic solvent systems. Acidic solvent systems gave round spots with better resolution of the components, whereas the basic solvent systems often gave elongated spots and the resolution of the

TABLE I

MOBILITIES OF CONJUGATED BILE ACIDS IN DIFFERENT SOLVENT SYSTEMS RELATIVE TO THAT OF TAUROCHOLIC ACID

The following compositions of the various solvents were used: $A_1$, chloroform–isopropanol–acetic acid–water (30:30:4:1); $A_2$, *n*-butanol–acetic acid–water (20:4:3); $A_3$, ethyl acetate–methanol–acetic acid–water (35:12:2:2); $N_1$, *n*-butanol–water (20:3); $N_2$, ethyl acetate–methanol–water (35:12:2); $B_1$, *n*-butanol–pyridine–water (20:4:3); $B_2$, ethyl acetate–methanol–pyridine–water (35:12:5:2).

| *Compound* | *Solvent system* | | | | | | |
|---|---|---|---|---|---|---|---|
| | $A_1$ | $A_2$ | $A_3$ | $N_1$ | $N_2$ | $B_1$ | $B_2$ |
| Taurolithocholic acid | 4.40 | 1.47 | 1.60 | 1.52 | 1.22 | 1.17 | 1.19 |
| Taurochenodeoxycholic acid | 2.50 | 1.28 | 1.36 | 1.36 | 1.12 | 1.11 | 1.12 |
| Taurodeoxycholic acid | 2.20 | 1.28 | 1.36 | 1.32 | 1.12 | 1.11 | 1.12 |
| Glycolithocholic acid | 13.2 | 2.10 | 1.86 | 1.14 | 0.84 | 0.88 | 0.84 |
| Glycochenodeoxycholic acid | 10.5 | 1.76 | 1.62 | 0.94 | 0.67 | 0.79 | 0.73 |
| Glycodeoxycholic acid | 9.5 | 1.76 | 1.62 | 0.90 | 0.67 | 0.79 | 0.73 |
| Glycocholic acid | 5.20 | 1.55 | 1.26 | 0.64 | 0.51 | 0.70 | 0.56 |
| Mobility of taurocholic acid (cm) | 1.0 | 5.8 | 8.3 | 5.0 | 10.7 | 8.1 | 11.2 |

various components was generally poor. Nevertheless, the basic systems had the advantage of changing dramatically the relative mobilities of the different conjugated bile acids. They showed a clear separation between the glycine and the taurine conjugated bile acids, the latter moving faster.

Separation between the taurine or glycine conjugates of chenodeoxycholic acid and deoxycholic acid was found to be difficult in a single development. System $A_1$ separated all four, but the two taurine conjugates were separated only partially (Table I and Fig. 1a). In order to achieve complete separation of these two acids, it was necessary to develop the plate twice in the solvent system ($A_1$) (Fig. 1b). (For a radio-assay, a third development in system $A_1$ was found to be more desirable.) However, it was easy to differentiate between the conjugated chenodeoxycholic and deoxycholic acids on the TLC plate since, when the plate was sprayed with 20% sulfuric acid after development and heated at 110° for 5 min, the conjugates of chenodeoxycholic acid appeared as brown spots whereas those of deoxycholic acid appeared as yellow spots.

The application of solvent system $A_1$ in resolving the components of human and guinea pig biles is illustrated in Figs. 2a and b. The major bile acid in guinea pig bile appears to be glycochenodeoxycholic acid, and a smaller amount of taurochenodeoxycholic acid was also observed[13]. Human bile appears to contain glycocholic, glycochenodeoxycholic and taurocholic acids, and small amounts of taurine conjugates of chenodeoxycholic and deoxycholic acids[14].

Recently, Goswami and Frey[11] described a solvent system consisting of chloroform–isopropanol–isobutanol–acetic acid–water (30:20:10:2:1) which could separate the taurine and glycine congates of deoxy- and chenodeoxycholic acids and the $R_F$ values of the various conjugated bile acids were comparable to those obtained in the present study. However, the system $A_1$ required only two runs of the TLC plate for a complete separation of these four conjugates, whereas Goswami and Frey had to use as many as six runs. System $A_1$ has also the advantage of exhibiting a group separation of the various glycine conjugated bile acids from their taurine conjugates.

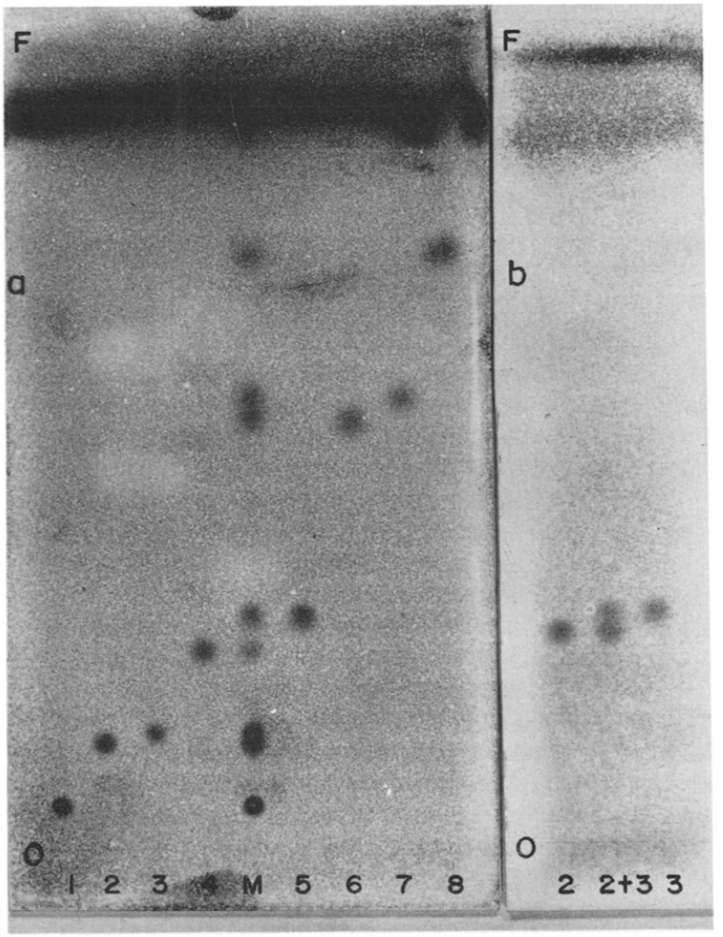


Fig. 1(a) Separation of the conjugated bile acids. Solvent system $A_1$ (Table I). O = Origin; 1 = taurocholic acid; 2 = taurodeoxycholic acid; 3 = taurochenodeoxycholic acid; 4 = taurolithocholic acid; M = mixture of conjugated bile acids; 5 = glycocholic acid; 6 = glycodeoxycholic acid; 7 = glycochenodeoxycholic acid; 8 = glycolithocholic acid; F = solvent front. (b) Separation of taurodeoxycholic acid and taurochenodeoxycholic acid. Solvent system $A_1$ (Table I); developed twice. O = Origin; 2 = taurodeoxycholic acid; 3 = taurochenodeoxycholic acid; F = solvent front.

In system $A_1$ and in systems described earlier[1,2,9–12], the glycine conjugates always moved faster than the corresponding taurine conjugates. When the proportion of acetic acid in the solvent system was reduced, the mobilities of all the conjugated bile acids decreased, but the effect was more pronounced on the glycine conjugates. On eliminating acetic acid completely from the solvent system (system $N_1$, Table I), the ratio of the mobilities of taurolithocholic acid, the least polar among the taurine conjugates, increased from 1:1 in the acidic solvent system $A_2$ to 2.4:1 in system $N_1$ (Figs. 3a and b). On the other hand, the ratio of the mobilities of glycolithocholic acid, the least polar glycine conjugate, and taurocholic acid, the most polar taurine conjugate, was reduced from 2.1:1 in system $A_2$ to 1.1:1 in system $N_1$ (Figs. 3a and b).

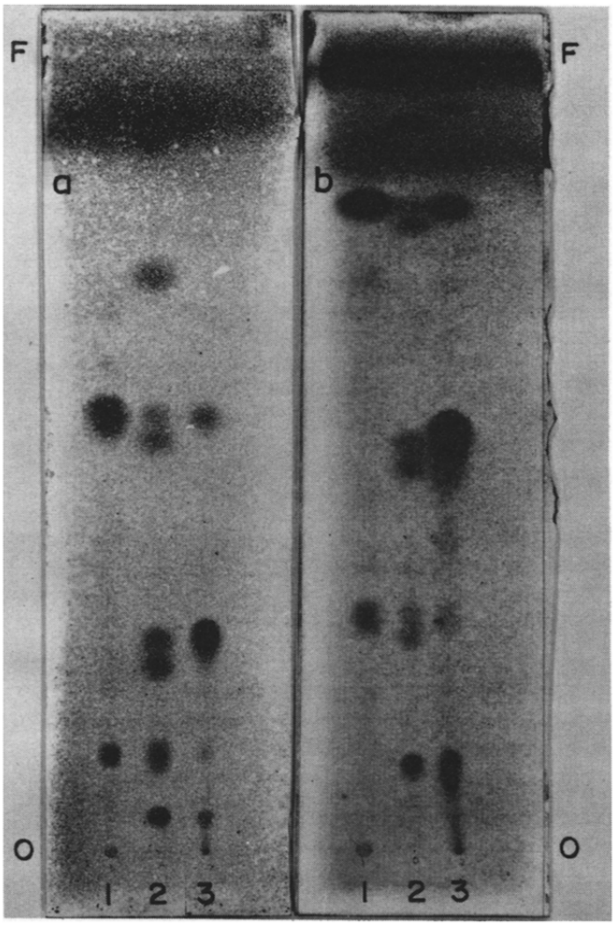


Fig. 2. Separation of biliary conjugated bile acids. Solvent system $A_1$ (Table I). O = Origin; 1 = guinea pig bile; 2 = mixture of standard bile acid conjugates (M, Fig. 1a); 3 = human bile; F = solvent front. (a) Developed once; (b) developed twice.

The mobilities of the conjugates of the two classes were completely reversed when acetic acid was replaced by pyridine (system $B_1$, Table I), all the taurine conjugated bile acids moved faster than any of the glycine conjugated ones (Table I). As is seen in Fig. 3c, taurolithocholic acid moved 1.7 times faster than glycocholic acid, whereas taurocholic acid moved 1.1 times faster than glycolithocholic acid. A similar dramatic change in the mobilities of the various conjugated bile acids was observed in solvent systems $A_3$, $N_2$ and $B_2$. Here, the reversal of the mobilities of the taurine and the glycine conjugates was observed even in the neutral system $N_2$.

It is thus evident that a combination of the acidic and the basic solvent systems can be used to completely separate and analyze a mixture of the taurine and glycine conjugates of lithocholic, deoxycholic, chenodeoxycholic and cholic acids. In addition, the change of pH from acidic to basic in the solvent system can serve as a criterion to establish more positively if a given conjugated bile acid belongs to the taurine or the glycine class.

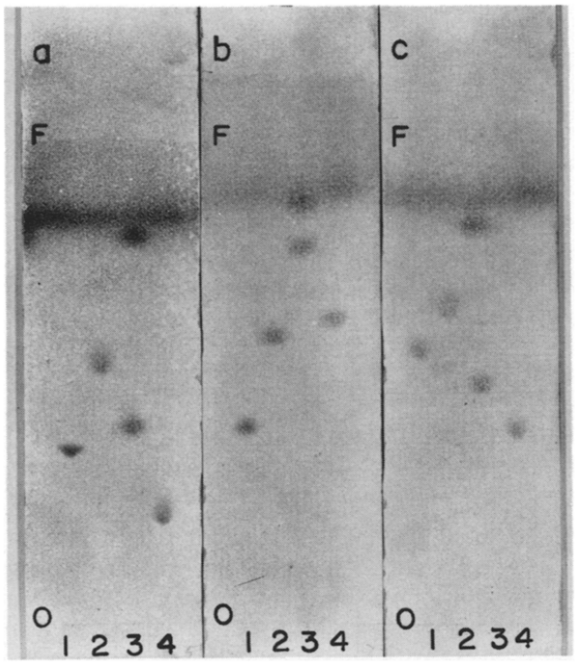


Fig. 3. Thin-layer chromatogram of conjugated bile acids. O = Origin; 1 = taurocholic acid; 2 = taurolithocholic acid; 3 = glycolithocholic acid (upper spot = lithocholic acid); 4 = glycocholic acid; F = solvent front. Solvent systems: (a) $N_1$ (Table I); (b) $A_2$; (c) $B_1$.

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