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Note

A specific reaction for chenodeoxycholic acid

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Thin-layer chromatography (TLC) and paper chromatography of bile acids are often used in clinical laboratories, although for quantitative and qualitative analysis gas chromatography is the method of choice^{1–3}. For the detection of bile acids in TLC, a phosphomolybdic acid reagent is often used^{4–6}.

Many specific reactions of bile acids have been described^{7,8}, the most specific for chenodeoxycholic acid being a reaction with ammonium ceric sulphate reagent⁸.

A reaction specific for chenodeoxycholic acid in the test-tube was first described by Wieland and Revery⁹. Chenodeoxycholic acid gives a cherry-red colour with acetic anhydride and sulphuric acid (the Liebermann–Burchard reagent). The same reagent was later used by Anthony and Beher⁷, but the colour given by chenodeoxycholic acid as described by these authors was not red and not useful in clearly differentiating between chenodeoxycholic and deoxycholic acids⁸. Here, we report our experiences in differentiating between the bile acids mentioned above.

METHODS AND RESULTS

In TLC different solvent systems^{4–6} and pre-coated plates [*e.g.*, Silufol (Kavalier) and DC Fertigplatten Kieselgel ohne Fluoreszenzindikator (Merck)] were used. The detection reagent was the same as reported previously^{7,8} (acetic anhydride containing 10% of sulphuric acid), and the plates were dried at room temperature before being sprayed.

The colour given by chenodeoxycholic acid was deep red; deoxycholic acid and cholic acid both gave yellow spots, and lithocholic acid gave no colour.

The red colour of chenodeoxycholic acid was stable for approximately 30 min, and the reaction was specific for both the free and the conjugated acid. The smallest amount that could be visually detected was 2 μg .

We have used this method many times in analyses of samples of chenodeoxycholic acid and in analysing human and rat bile for bile acids. The specific red colour of chenodeoxycholic acid observed by us was different from that described by Anthony and Beher⁷ and by Sundaram and Sodni⁸; the difference is probably due to different experimental conditions, especially in temperature during the reaction.

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