We wish to thank Professor EH Cooper of the Unit for Cancer Research and Dr D Norfolk of the Department of Haematology, Leeds General Infirmary, for their advice. M Bowen is supported by a grant from the Yorkshire Cancer Research Campaign and T Müller by the Swiss Cancer League.

References


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Measurement of conjugated and unconjugated serum bile acid concentrations using 3α-hydroxysteroid dehydrogenase

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Many reports have highlighted the importance of distinguishing between conjugated and unconjugated serum bile acids and thus have revived interest in earlier studies. Serum bile acids may be separated into their unconjugated and conjugated fractions by the method of Alme et al. Serum extracts are first subjected to cation exchange (with Amberlyst A-15) and the bile acids are then separated using the lipophilic anion exchanger diethylaminoethoxypropyl Sephadex LH-20 (Lipidex-DEAP). Once separated, the bile acids can be measured using gas-liquid chromatography, or radioimmunoassay. However, when we applied the hydroxysteroid dehydrogenase-fluorimetric assay for bile acids we obtained absurdly high recoveries from serum samples. This interference appeared to be associated with the use of Amberlyst A-15. When SP-Sephadex was substituted for Amberlyst A-15, no interference with subsequent enzyme analysis was observed.

Material and methods

Serum (usually 2 ml aliquots) was diluted with 0·1 M NaOH (10 ml) and methanolic extracts were prepared using either XAD-2 and the batch procedure or, recently, 0·01 M NaOH and Sep-Pak-C18 cartridges. An aliquot of the methanolic extract was kept for direct analysis of total 3α-hydroxy bile acids and the remainder evaporated to dryness. The residue was dissolved in 72% ethanol (vol/vol 2·5 ml) and applied to a Pasteur pipette column of SP-Sephadex (Sigma Chemical Co; 50 mm × 5 mm [internal diameter], taken to the H+ form with 0·05 M HCl in 72% ethanol). The eluate from the SP-Sephadex column was allowed to flow directly on to the Lipidex-DEAP column (Packard Instrument Ltd; 18 mm × 12 mm [internal diameter]; prepared as described). A further 2·5 ml 72% ethanol was added to the SP-Sephadex and the eluate again allowed to flow directly on to the Lipidex-DEAP column. The SP-Sephadex column was then discarded. The Lipidex-DEAP column was then eluted with the following solutions, prepared as described by Alme et al., each eluate being collected separately before the next solution was applied:

(i) 72% ethanol, 5 ml; (ii) 0·05 M acetate pH 3·8, 3 ml;
(iii) 0·1 M acetate pH 3·8, 1 ml; (iv) 0·1 M acetate pH 3·8, 2 ml;
(v) 0·3 M acetate pH 5·0, 3 ml; (vi) 0·3 M acetate pH 9·6, 3 ml. Eluates 2 and 3 were pooled, as were 4, 5, and 6. Each pooled fraction was evaporated to dryness and the residue dissolved in methanol. The methanolic extracts were then analysed using the enzymatic fluorimetric technique. The pooled fraction of eluates 2 and 3 contained the unconjugated bile acids, that of eluates 4, 5, and 6, the conjugated bile acids.

Results and discussion

During development of the method, separation was evaluated using standard solutions and labelled bile acids (14C-cholate, 13C-glycocholate, 14C-taurocholate and 3H-glycochenodeoxycholate). The recoveries of labelled and non-labelled bile acids from both aqueous solutions and serum samples that were taken through the entire procedure were high with negligible quantities appearing in other fractions (Tables 1 and 2). The results in 18 healthy subjects (laboratory personnel, nine women) were: fasting total 3α-hydroxy bile acids, 9·1 μmol/l ± 3·5 (mean ± SD; range 3·9–16·8 μmol/l), the corresponding unconjugated bile acid fraction was 32·4% ± 18·1
total bile acids (range 14·3–51·5%). Sulphated bile acids cannot be estimated by this procedure. These values for non-sulphated bile acids, however, are similar to those obtained by other workers using more sophisticated and time-consuming techniques such as gas chromatography—mass spectrometry, liquid chromatography—enzymatic fluorimetry, and thin layer chromatography—radioimmunoassay. This method provides a simple, convenient, and reliable technique for the separate determination of conjugated and unconjugated serum bile acids.

GM Murphy is supported by the Wellcome Trust.

References


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Letters to the Editor

Esterase staining in monocytes

In the classification of acute leukaemia non-specific esterase stains are helpful in discriminating between myeloblastic and monoblastic groups. The α-naphthyl butyrate esterase stain has been claimed by Li et al to be the most valuable for this purpose. Our routine stain has been α-naphthyl acetate esterase and before substituting the butyrate technique we evaluated both stains. Three observers studied the percentage positivity after counting 500 monocytes in the buffy coat of four healthy staff members at intervals up to 14 days from sampling to staining. Because we regularly receive air-dried non-fixed material from elsewhere we also varied the day of fixation.

Staining methods were according to Ornstein et al for the butyrate technique and for the acetate technique, the preparation was as follows:

The substrate solution contained...
valuable review of the present knowledge of platelet physiology and pathophysiology. Further he has described the effect of many drugs on platelet function and the use of these drugs in certain large clinical trials in cerebrovascular and ischaemic heart disease and in other specific clinical situations—for example, thrombotic thrombocytopenic purpura. Dr. Weiss has done a fantastic task in reviewing some 827 papers in 107 pages of text. This monograph is valuable and of wide interest and in spite of this condensed mass of fact it is interesting to read and still contains a significant amount of the author’s own wisdom and valuable criticism.

This monograph must be a valuable addition to the shelves of any Haematology Department or of any clinician who has an interest in the field of haemostasis or thrombosis. It is good value at the quoted price.

**Some new titles**

The receipt of these books is acknowledged and this listing must be regarded as sufficient return for the courtesy of the lender. Books that appear to be of particular interest will be reviewed as space permits.


**Medical Microbiology Laboratory Procedures.** Lucy Treagan and Lynn Pulliam. (Pp 318; illustrated; £12.) WB Saunders Company. 1982.

**Progress in Clinical Cancer.** Vol VIII. Ed Irving M Ariel. (Pp 350; $74.50.) Grune & Stratton Inc. 1982.


**Correction**

In the paper by Smith *et al*1 in the February 1983 issue, page 235, Material and methods, lines 22–24 should read:

(i) 72% ethanol, 5 ml; (ii) 0·15 M acetate pH 3·8, 3 ml; (iii) 0·3 M acetate pH 3·8, 1 ml; (iv) 0·3 M acetate pH 3·8, 2 ml; (v) 0·9 M acetate pH 5·0, 3 ml; (vi) 0·9 M acetate pH 9·6, 3 ml.

The authors apologise for this error which was due to a mistake in the calculation of the molarity of a stock solution of acetic acid.

**Reference**