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

- ¹ Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radialimmunodiffusion. *Immunochemistry* 1965;2:235-54.
- ² Vaheri A, Vartio T, Stenman S, Saksela O, Hedman K, Alitalo K. Fibronectin and proteinases in tumor invasion. In: Strauli P, Barrett AJ, Baici A, eds. *Proteinases and tumor invasion*. New York: Raven Press, 1980:49-57.
- ³ Mosesson MW, Amrani DL. Review: the structure and biologic activities of plasma fibronectin. *Blood* 1980;56:145-58.
- ⁴ Mollison PL. Blood transfusion in clinical medicine. 6th ed. Oxford: Blackwell Scientific Publications, 1979.
- ⁵ Pearlstein E, Gold LJ, Garcia-Pardo A. Fibronectin: a review of its structure and biological activity. *Mol Cell Biochem* 1980;29:103-28.

Requests for reprints to: Mrs Margaret Bowen, The Unit for Cancer Research, School of Medicine, Leeds LS2 9NL, England.

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

SM SMITH, M MYSZOR, KDR SETCHELL,* GM MURPHY *Gastroenterology Unit, Guy's Hospital and Medical School, and the *Division of Clinical Chemistry, Clinical Research Centre, Harrow*

Many reports have highlighted the importance of distinguishing between conjugated and unconjugated serum bile acids¹⁻⁵ and thus have revived interest in earlier studies.^{6,7} Serum bile acids may be separated into their unconjugated and conjugated fractions by the method of Alme *et al.*⁸ Serum extracts are first subjected to cation exchange (with Amberlyst A-15) and the bile acids are then separated using the lipophilic anion exchanger diethylaminohydroxypropyl Sephadex LH-20 (Lipidex-DEAP). Once separated, the bile acids can be measured using gas-liquid chromatography,⁵ or

radioimmunoassay.⁹ However, when we applied the hydroxysteroid dehydrogenase-fluorimetric assay for bile acids¹⁰ 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-15,¹¹ 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 procedure¹² or, recently, 0.01 M NaOH and Sep-Pak-C₁₈ cartridges.¹³ An aliquot of the methanolic extract was kept for direct analysis of total 3 α -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 $\times$ 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 $\times$ 12 mm [internal diameter]; prepared as described⁸). 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.*,⁸ 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, ¹⁴C-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 3 α -hydroxy bile acids, 9.1 μ mol/l $\pm$ 3.5 (mean $\pm$ SD; range 3.9-16.8 μ mol/l), the corresponding unconjugated bile acid fraction was 32.4% $\pm$ 18.1

Table 1 Recovery of labelled bile acids (mean (%) $\pm$ SD) through whole procedure

Bile acid	n	Overall recovery	Proportion (%)	
			Unconjugated	Conjugated
¹⁴ C-choleate acid (2303 DPM)	6	97.2 $\pm$ 2.6	99.8 $\pm$ 0.1	0.2 $\pm$ 0.1
¹⁴ C-glycocholate acid (1370 DPM)	6	97.5 $\pm$ 2.9	0.5 $\pm$ 0.6	99.5 $\pm$ 0.6
³ H-glycochenodeoxycholate (2571 DPM)	4	98.2 $\pm$ 2.9	4.7 $\pm$ 0.9	95.6 $\pm$ 0.7
¹⁴ C-taurocholate (790 DPM)	2	94.2 $\pm$ 2.1	0.9 $\pm$ 0.2	99.1 $\pm$ 0.3

Table 2 Recovery of standard bile acid solutions added to sera and taken through the whole procedure

Bile acid	n	Added (μ mol/l)	Estimated (μ mol/l)	Proportion (%)	
				Unconjugated	Conjugated
Chenodeoxycholate	4	13.6	13.0 $\pm$ 1.9	96.1 $\pm$ 3.6	3.9 $\pm$ 3.5
Lithocholate	4	11.1	11.1 $\pm$ 1.2	96.2 $\pm$ 3.8	3.9 $\pm$ 3.8
Glycochenodeoxycholate	4	12.1	13.1 $\pm$ 2.4	2.9 $\pm$ 3.5	96.9 $\pm$ 3.4
Glycocholate	4	8.4	9.0 $\pm$ 1.4	4.6 $\pm$ 6.0	95.5 $\pm$ 6.0
Tauroolithocholate	4	12.4	12.4 $\pm$ 0.5	3.5 $\pm$ 3.1	96.6 $\pm$ 3.1

total bile acids (range 14.3–51.5%). Sulphated bile acids can not 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;^{1–5} high pressure liquid chromatography—enzymatic fluorimetry;^{2–4} and thin layer chromatography—radioimmunoassay.³ 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

- Bremmelgaard A, Alme B. Analysis of plasma bile acids: profiles in patients with liver disease associated with cholestasis. *Scand J Gastroenterol* 1980;**15**:593–600.
- Kimura H, Suzuki N, Sato T, Goto J, Nambara T. Separatory determinations of free and conjugated bile acids in human serum. *Jpn J Clin Chem* 1979;**8**:126–30.
- Matern S, Titjen KE, Fackler O, Hinger R, Herz R, Gerok W. Bioavailability of ursodeoxycholic acid in man: studies with a radioimmunoassay for ursodeoxycholic acid. In: Paumgartner G, Stiehl A, Gerok W, eds. *Biological effects of bile acids*. Lancaster: MTP Press, 1979:109–18.
- Okuyama S, Kokubun N, Higashidate S, Uemura D, Hirata Y. A new analytical method of individual bile acids using high performance

- liquid chromatography and immobilised 3 α -hydroxysteroid dehydrogenase in column form. *Chem Lett (Japan)* 1979;**12**:1443–6.
- Satchell KDR, Lawson AM, Blackstock EJ, Murphy GM. Diurnal changes in serum unconjugated bile acids in normal man. *Gut* 1982;**23**:637–42.
- Makino I, Nakagawa S, Mashimo K. Conjugated and unconjugated serum bile acid levels in patients with hepatobiliary diseases. *Gastroenterology* 1969;**56**:1033–9.
- Lewis B, Panvelwalla D, Tabaqchali S, Wootton IDP. Serum bile acids in the stagnant loop syndrome. *Lancet* 1969;**i**:219–20.
- Alme B, Bremmelgaard A, Sjøvall J, Thomassen P. Analysis of metabolic profiles of bile acids in urine using a lipophilic anion exchange and computerised gas-liquid chromatography—mass spectrometry. *J Lipid Res* 1977;**18**:339–62.
- Myszor MF, Smith SM, Murphy GM, Dowling RH. A radioimmunoassay for unconjugated and conjugated ursodeoxycholic acid in normal sera. *Clin Sci* 1982;**62**:50p.
- Murphy GM, Billing BH, Baron DN. A fluorimetric and enzymatic method for the estimation of serum total bile acids. *J Clin Pathol* 1970;**23**:594–8.
- Satchell KDR, Matsui A. Serum bile acid analysis. *Clin Chim Acta* 1982; (in press).
- Barnes S, Chitranukroh A. A simplified procedure for the isolation of bile acids from serum based on a batch extraction with the non-ionic resin—amberlite XAD-7. *Ann Clin Biochem* 1977;**14**:237–9.
- Satchell KDR, Worthington J. A rapid and quantitative method for the extraction of bile acids from serum using commercially available reverse phase octadecylsilane bonded silica cartridges. *Clin Chim Acta* 1982; (in press).

Requests for reprints to: Dr GM Murphy, Gastroenterology Unit, 18th Floor, Guy's Tower, Guy's Hospital and Medical School, London SE1 9RT, England.

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.¹

Our routine stain has been α -naphthyl acetate esterase and before substituting the "butyrate technique" we evaluated both stains. Three observers studied the percentage positively 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*² 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.

AA SHARP

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.

Development of Cancer Centres and Community Cancer Control Programmes. Report on a WHO Working Group. EURO Reports and Studies 70. (Pp 38; paperback; Sw fr 4.) World Health Organisation. 1982.

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.

Mutagens in our Environment. Progress in Clinical and Biological Research. Vol. 109. Ed Marja Sorsa and Harri Vainio. (Pp 504; £38.) Alan R Liss 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

- ¹ Smith SM, Myszor M, Setchell KDR, Murphy GM. Measurement of conjugated and unconjugated serum bile acid concentrations using 3 α -hydroxysteroid dehydrogenase. *J Clin Pathol* 1983;**36**:235–6.