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.

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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.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

Sørensen phosphate buffer according to Dacie and Lewis;³ (ii) 1.2 ml hexazolyised pararosaniline; (iii) 1.0 ml α -naphthyl acetate solution (20 mg α -naphthyl acetate in 1.0 ml ethylene glycol monomethylether.

After fixation of the air-dried smears in fixative solution (phosphate-buffered saline with 45% acetone and 25% formaldehyde (36%); pH 6.6) for 30 s, the smears were washed in distilled water, then immersed in freshly prepared substrate at 37°C for 45 min and then rinsed in distilled water. The smears were counterstained for 10 min with haematoxylin and then rinsed again in distilled water.

Table 1 shows that the results of the butyrate technique are influenced by the time intervals to both fixation and staining. Although not shown in Table 1, interobserver variation was large (3-41%).

The acetate technique was found to be reproducible and the intensity of staining persisted even when fixation was delayed for as long as 14 days (Table 2). Discrepancy between observers was negligible. In conclusion the butyrate technique does not seem to be a reliable discriminant for the differentiation of monocytes in material that cannot be processed immediately. Even if processing is immediate the "butyrate" staining still seems to be less sensitive than "acetate" staining; in addition, discrepancy between observers is considerable with butyrate esterase staining.

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Table 1 Mean percentage of monocytes positive in the α -naphthyl butyrate esterase stain in buffy coat of four healthy volunteers examined by three observers.

Volunteer	Fixation on day of receipt		
	Staining (days after receipt)		
	0	2	8
1	83	73	51
2	60	10	33
3	90	90	85
4	41	23	27
	Fixation and staining on same day		
	Days after receipt		
	0	2	8
1	83	34	55
2	61	8	15
3	89	62	61
4	40	21	25

Table 2 Mean percentage of monocytes positive in the α -naphthyl acetate esterase stain in buffy coat of four healthy volunteers, examined by three observers.

Volunteer	Fixation and staining on same day		
	Days after receipt		
	0	7	14
1	99	98	98
2	95	95	95
3	94	94	94
4	98	95	95

Tyrosine crystals in a parotid pleomorphic adenoma in a Vietnamese boat person

In the recent paper by Thomas and Hutt¹ and the recent letter by Friedmann *et al*² crystalline deposits of tyrosine were described in pleomorphic adenomas of salivary glands in ceruminous gland tumours. Thackray and Lucas³ also reported tyrosine crystalline deposits in pleomorphic adenomas of salivary glands. Reference has been made in these articles to the relatively high frequency of tyrosine crystal deposition in black patients. Only a few caucasian patients have been found with tyrosine crystals present in salivary gland tumours. Loke,⁴ in a report from Malaya, did not report the phenomenon of tyrosine deposition in salivary gland tumours in Malays, Chinese, and Indians. This letter notes tyrosine crystal deposition in a pleomorphic adenoma in an Indochinese patient.

The patient was a 60-year-old Chinese woman who left Vietnam by boat in 1980 and arrived as a refugee in Darwin, Australia, after several months at sea. After her arrival in Australia she remained in Darwin and her health was good. Two years later she presented with a mobile

mass anterior to the right angle of the mandible. This mass had been present for at least three years. A right superficial parotidectomy was performed, and within the resected tissue a well defined ovoid grey tumour, measuring 1.5 cm $\times$ 1 cm $\times$ 1 cm, was found. On section the tumour had a blue-grey appearance. This histology was typical of a pleomorphic adenoma. There were many myxochondroid areas. In these areas numerous eosinophilic, flower-shaped crystalline structures were seen (Figure). These crystals gave a positive

Multiple flower-shaped crystals of tyrosine in myxochondroid region of pleomorphic adenoma of parotid. Haematoxylin and eosin $\times$ 220

reaction with Millon's stain, indicating tyrosine.

Tyrosine crystal deposition in salivary gland pleomorphic adenomas is an unexplained phenomenon, though it may be that the deposition of crystals may be related to the long duration of the tumour.

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