Table

Total No of specimens examined by histology and microbiology	33
Gimenez positive (light microscopy)	18 (54·5%)
Gram positive (microbiology)	17 (51·5%)
Culture positive (microbiology)	16 (48%)
Culture positive and Gimenez negative	3
Culture negative and Gimenez positive	4
Gram positive and Gimenez negative	2
Gram negative and Gimenez positive	6
Concordance between:	
Gram, culture, and Gimenez	24 (72%)
Culture and Gimenez	26 (79%)
Gram and Gimenez	25 (76%)

1964 by Gimenez.[4] Greer[5] used this method for identification of *Legionella pneumophilia* and found that it was successful in direct staining of lung smears in paraffin sections. This was attributed to processing, but it may be due to the site of the *Legionella* bacteria, which are intracellular. We found that the Gimenez technique was satisfactory both in smears and paraffin sections of necropsy lung with *L pneumophilia* and other bacterial infections, apparently staining all types of bacteria (McMullen L, personal observation).

The Wartin-Starry stain, a recognised technique for the identification of spiral bacteria,[6] is time consuming, expensive, and technically elaborate. The results can be unreliable due to silver precipitate, and difficulty arises in the identification of separate elements.

The Gram method, although successful in direct smears of the biopsy specimen, presented problems if only a few bacteria were present. In paraffin sections the Gimenez technique was found to be superior.

To detect *Campylobacter* in biopsy specimens of the gastrointestinal tract both bacteriology and histology should be used. The good results, however, obtained with the Gimenez method will allow studies to be performed on retrospective series.

In studies of gastric antral mucosa we found that the Gimenez technique was quick, economical, and reliable in the detection of *Campylobacter* sp in paraffin sections.

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Simple immunoturbidimetric method for determining urinary albumin at low concentrations using Cobas-Bio centrifugal analyser

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The finding that a raised concentration of urinary albumin, undetectable by Albustix (Ames Co Ltd, Stoke Poges, Slough, UK)—that is, microalbuminuria predicts potentially reversible nephropathy[1–5]—has led to an increasing demand from clinicians for this determination. Microalbuminuria has been strictly defined as an albumin excretion rate of $>15\,\mu g$/minute (Albustix negative sample) in three major studies.[1–3] Assay methods should be specific for albumin and sufficiently sensitive down to at least 3 mg/l to cover the low concentrations that may be found in cases of microalbuminuria. Existing techniques include radioimmunoassay,[6] radial immunodiffusion,[7] enzyme linked immunosorbent assay,[8] and nephelometry.[9] Because they may be time consuming, costly, or require large batch sizes, an alternative approach has been sought based on immunoturbidimetry and use of the Cobas-Bio centrifugal analyser.

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Material and methods

The assays were performed using the Cobas-Bio (version 8144) centrifugal analyser (Hoffman La Roche, Basle, Switzerland).

REAGENTS

Phosphate buffered saline (PBS) at pH 7·4 (50 mmol/l).

Disodium hydrogen phosphate (5·22 g), 1·33 g of potassium dihydrogen phosphate, 5·8 g of sodium chloride, and 1·0 g of sodium azide were dissolved in distilled water, the pH was adjusted to 7·4 with 12 mol/l hydrochloric acid, and the volume made up to 1 litre.

Buffered polyethylene glycol (5% w/v)

Polyethylene glycol 6000 (PEG) (Technicon, Basingstoke, UK) (50·0 g) was dissolved in and made up to 1 litre with PBS.

Antisera

Antisera to human albumin was obtained from the department of immunology, Royal Hallamshire Hospital, Sheffield. This was subjected to immunoelectrophoresis and found to be specific for human albumin. One volume of the stock solution was diluted with 120 parts of buffered PEG.

Albumin standards

Purified human albumin (100 mg) (Behring Instut, West Germany) was dissolved in 200 ml of PBS. This stock standard (500 mg/l) was stored in aliquots of 2 ml at −20°C. Working standards were prepared fresh at the time of assay by diluting the stock standard in PBS to cover the assay range 2–100 mg/l.

URINE SAMPLES

All urine samples were pretested with Albustix (Ames Co Ltd, Stoke Poges, Slough), and those giving a positive result—that is, trace or greater—were diluted with PBS into the assay range. All samples were centrifuged for 10 minutes at 2000 rpm before assay to remove any debris that might interfere with absorbance readings.

ASSAY PROCEDURE

Aliquots of urine samples and standards were pipetted into Cobas-Bio sample cups, which were placed on the sample disc. A cuvette rotor was installed and the disc placed on the sample module. The assay was performed with the instrument settings shown in table 1.

STUDIES PERFORMED

Optimum PEG concentration

The effect of PEG 6000 on the enhancement of the antigen-antibody reaction was studied using PEG concentrations of 0, 4, 5 and 6% w/v. For this purpose the following albumin concentrations were used: 1, 5, 10, 15, 30, 80, 100 and 120 mg/l.

Table 1 *Cobas-Bio variable listing*

Urine albumin Alpha code		+
1	Units	mg/l
2	Calculation factor	1000
3	Standard 1 CONC	2
3	Standard 2 CONC	10
3	Standard 3 CONC	20
3	Standard 4 CONC	50
3	Standard 5 CONC	80
3	Standard 6 CONC	100
6	Limit	0
7	Temperature (°C)	25
8	Type of analysis	7·5*
9	Wavelength (nm)	340
10	Sample volume (ul)	20
11	Diluent volume (ul)	30
12	Reagent volume (ul)	200
13	Incubation time (seconds)	0
14	Start reagent volume (ul)	0
15	Time of first reading (seconds)	5·0
16	Time interval (seconds)	180
17	No of readings	2
18	Blanking mode	1
19	Printout mode	1

*Code for semikinetic type reaction using the DENS (Data Evaluation of non-linear standard curves) option with automatic printout of results.

Optimum antibody dilutions

Having chosen the most suitable PEG concentration, the optimum dilution for the antibody was found using antibody dilutions of 1/101, 1/121, and 1/141 with the same range of albumin standards. These antibody dilutions were chosen because the 1/101 dilution was already in use for serum albumin determination by a similar immunoturbidimetric method, based on that of Wenham and Horn.[10]

Reproducibility and accuracy

Intrarun precision was found by assaying 19 replicates of five different urines. Day to day precision was assessed by using five different urines on 20 separate occasions. Recovery was determined by adding different amounts (10·2, 25·6, 56·3, 82·1 mg/l) of purified human albumin to a urine pool.

Comparison of methods

The proposed immunoturbidimetric method was compared with an established radioimmunoassay[6] by analysing 193 urines from diabetic patients with and without microalbuminuria. Results were compared statistically using the Pearson's correlation coefficient and linear regression analysis.

Results and discussion

Fig 1 shows the standard curves obtained with different PEG concentrations. In the absence of PEG there was very little reaction. There were two disadvantages to the incorporation of 4% PEG: the

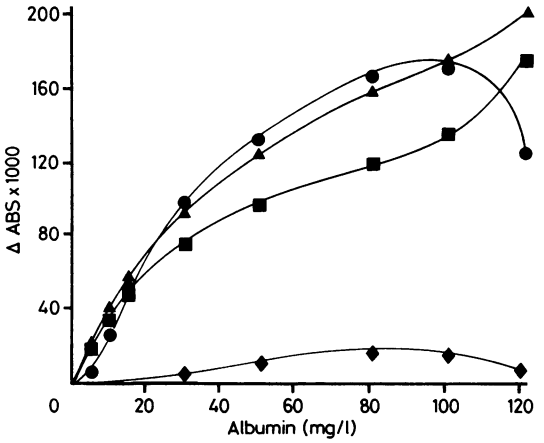

Fig 1 *Albumin standard curve using various PEG concentrations (◆–0% PEG; ●–4% PEG; ▲–5% PEG; ■–6% PEG).*

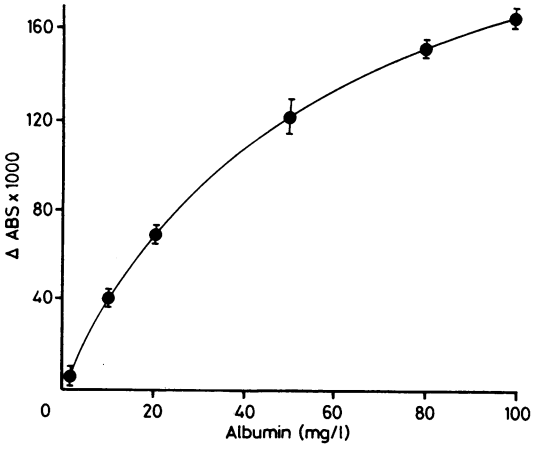

Fig 2 *Typical albumin standard curve showing +/− 2 standard deviations (n = 30).*

difficulty in detecting antigen excess at concentrations of urinary albumin greater than 90 mg/l; and at albumin concentrations less than 15 mg/l the curve shape was difficult to fit using mathematical models. The standard curve obtained with 6% PEG was insensitive at albumin concentrations greater than 30 mg/l, making it unsuitable for use in this assay. Maximum sensitivity over the assay range, however, was obtained with 5% PEG, albumin values could be measured up to 100 mg/l, and a smooth curve was obtained at concentrations below 15 mg/l. The unusual shape of the curve between 100 and 120 mg/l has also been observed with higher PEG concentrations of 7% and 8% (data not shown) and with other proteins. This phenomenon, which we called the "flip" effect, is under further investigation. Values read off this part of the curve will be printed out as greater than the top standard (100 mg/l). Albumin concentrations of 150 mg/l or more should have been detected as "trace" on Albustix and the sample diluted. Hence the use of 5% PEG in the assay gives a confident prediction that the result is greater than 100 mg/l and requires repeating on a dilution of the urine.

Standard curves using three different dilutions of antibody were similar. The 1/101 and 1/121 antibody dilution gave similar standard curves, that of 1/141 was slightly less sensitive, and for economical reasons the 1/121 dilution was therefore used. The antibody dilution used depends on its titre, which will vary from batch to batch. It is therefore important to find the appropriate dilution for each new batch of antisera. Fig 2 shows a typical standard albumin curve (2–100 mg/l) using reagents at the selected optimum and shows that albumin can be measured down to at least 2 mg/l. The reproducibility for 30 standard curves is also indicated.

Table 2 shows data for precision and recovery. Intrabatch precision was less than 3·3%, interbatch precision less than 4%. Recoveries of 96% to 103% were obtained over the assay range.

The results for the urines from the diabetic patients assayed by this technique and by radioimmunoassay showed good agreement; r = 0·99; y = 1·03x − 0·37.

In summary, the immunoturbidimetric method described was sensitive (at least to 2 mg/l), precise, and accurate, with a high degree of specificity. It is an economical assay, both in terms of reagent costs (2 pence per test), and Cobas-Bio running time, which was about eight minutes for a tray of 19 samples.

Table 2 *Summary of data for precision and recovery experiments*

Intrabatch precision (n = 19) *Level*	*Mean mg/l*	*SD*	*% CV*
1	4·9	0·16	3·3
2	16·4	0·21	1·3
3	33·6	0·48	1·4
4	62·2	1·30	2·1
5	89·2	1·28	1·4
Interbatch precision (n = 20)			
1	5·2	0·20	3·9
2	15·4	0·45	3·0
3	34·2	0·96	2·8
4	53·3	1·81	3·4
5	96·2	3·41	3·5
Recovery			
	Added mg/l	*Recovery %*	
1	10·2	96	
2	25·6	103	
3	56·3	102	
4	82·1	97	

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Letters to the Editor

Link between pulmonary megakaryocytes and cardiovascular and respiratory disease

We are delighted that the recent study of Sharma and Talbot[1] provides support for our original hypothesis[2] that pulmonary platelet production could be the "missing link" between cardiovascular and respiratory disease.

Although the presence of pulmonary megakaryocytes does not prove that the lungs are the only site of thrombopoiesis, the wealth of additional evidence cited in our earlier study[3] and reiterated by Sharma and Talbot[1] makes pulmonary platelet production seem highly likely. In particular, the number of megakaryocytes/ml of blood in the pulmonary artery needed to maintain a normal steady state platelet count[3] and the number observed in central venous blood of man[4] is of the same order of magnitude. Hence bone marrow production is not necessary for platelet or megakaryocyte homoeostasis. Furthermore, pulmonary thrombopoiesis allows a simple mechanism of platelet production to be invoked. The process of sequential random binary division of megakaryocyte cytoplasm provides a physical analogue of mitosis,[3] which can be simulated on a computer.[5] No quantitative evidence to support the theory of bone marrow thrombopoiesis has been proposed.

It is worth noting that the statistical comparisons based on the stereological procedure advocated by Sharma and Talbot[1] should be treated with caution. Larger three dimensional structures—for example, megakaryocytes with cytoplasm—are more likely to be sectioned than smaller structures (naked nuclei). Unless the appropriate correction factors are applied,[6] the counting procedure will be biased toward the larger structure. In addition, the total number of pulmonary megakaryocytes is also influenced by their size. A characteristic of the polyploid megakaryocyte is its ability to increase in size in response to stimulation,[7] resulting in an increased probability of pulmonary trapping.[8] Megakaryocytes are larger than normal in cardiovascular disease[9] and in experimental models of atherosclerosis.[10] Hence under these and similar pathological conditions, more pulmonary megakaryocytes are likely to be seen even though megakaryocyte counts in central venous blood may be normal. This may explain the pulmonary megakaryocyte count differences reported in hospital and forensic necropsies.[11]

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