Technical methods

Loctite 357 contains no solvent to evaporate, coverslip detachment was not a problem. Loctite 357, unlike other mounting media, did not at any time show crystalline or amorphous precipitation when examined using polarising microscopy. The combination of the substrate for the localisation of non-specific esterase was chosen because it has been our experience that this particular reaction product localises very well and has a high optical density, but its major drawback has been its solubility in hydrocarbon solvents. This problem has now been overcome as the reaction product is not soluble in unpolymerised or polymerised Loctite 357. No subjective loss of stain density or diffusion of stain has been noted in the Loctite embedded preparations when re-examined up to three years later. Sections stained for many other enzymes have been mounted and similar results obtained.

In this laboratory plastic resins are used only for embedding undecalcified bone for histomorphometric analysis. The mounting of stained or unstained plastic sections of this type using conventional mounting media can lead to unevenness of the section due to differential swelling of the tissue and the plastic embedding medium in the presence of the hydrocarbon solvent. Loctite 357 has no solvent base and does not therefore cause uneven swelling of the section. Achieving "flat" sections of undecalcified bone biopsies is therefore rendered simpler than with conventional mounting media.

The mounting method described has been used routinely for sections of undecalcified bone stained with the von Kossa reaction, acid solochrome azurine,² Masson Goldner, and other staining techniques. No loss of stain was noted on any of the sections that had been stored in the dark when examined at bimonthly intervals over the next year. Twenty micron unstained sections of undecalcified bone from patients that had received an *in vivo* label of ledermycin as a marker of calcification fronts were mounted in Loctite 357 in the same way. The polymerised resin is not itself fluorescent, nor does it mask the

tetracycline fluorescence. In addition, it does not decalcify the bone or remove the tetracycline from the section, even when the longest mounted slides are reviewed (at the time of writing, about three years).

Discussion

Particularly for mounting sections from resin embedded tissue and sections stained using immunocytochemical and enzymatic reactions, Loctite 357 has considerable advantages over conventional mounting media. It would be wrong to suggest that it is without any disadvantages, but the only problems encountered in this laboratory, where the techniques outlined above are in regular use by technical staff of all levels of experience, have been relatively minor. Only two could be regarded as noteworthy. Firstly, once the resin is polymerised it is impossible to remove the coverslip, and, secondly, we have found that on hot bright sunny days direct sunlight (even through closed windows) will polymerise the resin almost as rapidly as an artificial ultraviolet light source. Mounting is therefore undertaken away from direct sunlight but still in the routine laboratory. Loctite 357 is about 10 times the cost of XAM or DPX, but the small amount used (1000 slides can be mounted from a 50 ml bottle costing £20) compared with the advantages make this a minor problem.

In conclusion, Loctite 357 is an easily handled, multiuse mounting medium which, in certain circumstances, is superior to conventional plastic and solvent mountants.

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Sensitive and rapid measurement of C-reactive protein (CRP) by lipid agglutination

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C-reactive protein (CRP) is a pentameric molecule produced in the liver during the acute phase response to infection or tissue injury.¹⁻³ It was originally described as binding to the C-polysaccharide component of pneumococcus.⁴ Clinical studies have shown that a raised concentration of CRP in serum is an early predictor of bacterial infection in children,⁵ in patients with acute leukaemia,⁶ and in newborn infants.⁷ Its potential clinical usefulness has recently been re-emphasised.³

Hulman *et al*⁸ described an agglutination reaction between the sera of acutely ill patients and a fat emul-

sion, Intralipid (used for intravenous parenteral nutrition).⁸ They attributed the agglutination or "creaming" reaction to the presence of CRP in the sera.

We have developed a simple inexpensive lipid agglutination method for estimating raised concentrations of CRP in serum using a standard platelet aggregometer. This procedure is suitable for rapid screening for the presence of CRP in patients at high risk.

Material and methods

Nutralipid 10% (w/v) was bought from KabiVitrum (Sweden), and standard serum and antiserum to human CRP from Behringwerke (Marburg, West Germany). All other chemicals were reagent grade.

Agglutination reactions were performed in a dual channel platelet aggregometer (Payton Associates Ltd, Canada) linked to a chart recorder (Fisher Scientific, Canada). CRP was quantified using a laser nephelometer (Behringwerke, Marburg, West Germany). Serum samples referred to the haematology laboratory for CRP analysis were processed immediately or frozen at -70°C until tested.

AGGLUTINATION REACTION

To an aggregometer cuvette containing a stir bar, 500 μl Tris buffer (0.01 M Tris-(hydroxymethyl)-aminomethane, 0.14 M NaCl, 0.002 M CaCl_2 , pH 7.2) and 10 μl stock Neutralipid were added. The mixture was warmed to 37°C with constant stirring (9000 rpm). The transmittance was adjusted to 30% using a full scale deflection of 1 millivolt. After a baseline had been established 10 μl of the patient's serum or CRP standard serum was added to the mixture. The reaction was monitored for no less than five minutes.

CRP QUANTITATION

Estimation of concentrations of CRP in serum was performed as outlined by Behringwerke. Serum samples were diluted 1/75 and 1/150 in 0.15 M NaCl. Fifteen microlitres of CRP antiserum was added to 300 μl of each dilution in a nephelometry cuvette. The cuvettes were agitated gently, then incubated at room temperature for 60 minutes. Voltage readings were taken and compared with a standard curve constructed using known concentrations of CRP.

PATIENTS

Four newborn infants with systemic infection admitted to the neonatal intensive care unit had serial concentrations of CRP in serum measured simultaneously by the lipid agglutination reaction and by laser nephelometry.

STATISTICAL METHODS

Analysis included calculation of coefficients of variation (interrin and intrarin), and correlation coefficients were determined using linear regression analysis.

Results

The table shows the optimal conditions for CRP-Nutralipid agglutination were first established. As for CRP and liposome agglutination calcium ions were necessary for the reaction to occur.⁹ The presence of magnesium ions, up to physiological concentrations, had no effect on the agglutination reaction. Lipaemic sera, or sera containing rheumatoid factor, did not affect the agglutination response. We attributed this to the higher concentration of Neutralipid in relation to plasma lipid in the reaction mixture.

Fig 1 shows tracings of CRP-Nutralipid agglutination from 11 mg/l to 196 mg/l of CRP. This dilution series produced a correlation coefficient of 0.995 for a plot of CRP concentration *v* slope (inset). The initial

Table Optimal conditions for CRP agglutination

Variable	Measurement
pH	7.0-7.2
Ca^{++} activity	2 mM
Lipid concentration	2.0 g/l
CRP concentration	> 5.0 mg/l
Temperature	37°C

Fig 1 Reaction between dilution series of CRP in sera ($A = 196 \text{ mg/l}$, $B = 86 \text{ mg/l}$, $C = 43 \text{ mg/l}$, $D = 22 \text{ mg/l}$, $E = 11 \text{ mg/l}$) and constant amount of Neutralipid. 10 μl sera + 10 μl Neutralipid (10% w/v) + 500 μl Tris buffer. Inset: CRP concentration and aggregometer tracing. Slope = change in transmittance over time measured at steepest portion of ascending arm of tracing ($r = 0.995$).

rate of increase in light transmittance could be determined within five minutes of initiating the reaction.

Random serum samples were analysed for the presence of CRP using the agglutination reaction and laser nephelometry. Fig 2 shows a scattergram of CRP concentration, as determined by laser nephelometry, against agglutination slope. A good correlation exists between the two methods ($r = 0.954$). The reproducibility of the assay was tested between successive runs and within a run. The coefficients of variation were acceptable, both within a run (3.0%; $n = 11$) and between runs (6.5%; $n = 15$).

The raised CRP concentrations in the four neonates on admission (mean 52.1 mg/l) fell serially over 72 hours of treatment). Fig 3 shows a comparison of lipid agglutination slope and CRP concentration by laser nephelometry in an infected infant. The correlation coefficients comparing the two methods for each serial determination in the infants tested were >0.91 .

Discussion

A lipid agglutination reaction was used to detect the presence of CRP in sera. This reaction is based on the ability of CRP to bind to lipid vesicles in the presence of calcium ions. Previous work has shown that CRP binds with crude soybean phosphatidylcholine, a constituent of the fat emulsion in Nutralipid.⁹

Lipid emulsions used in parenteral nutrition are composed of lipid particles from 0.1 to 0.5 μm in diameter.⁸ An agglutination response similar to that

seen with platelet aggregation studies results when CRP is mixed with these lipid particles in the presence of calcium ions. An initial decrease in light transmission is seen, followed by a gradual rise caused by an increase in forward light scattering. The initial rate of change of increased light transmittance is proportional to the amount of CRP in the serum (fig 1).

Tests currently available for detecting CRP include latex agglutination,¹⁰ laser nephelometry,¹¹ radial immunodiffusion,¹² and electroimmunodiffusion.¹³ These tests all require polyclonal antisera to CRP.

Fig 3 Serial measurements of CRP concentration by nephelometry. (—●—) and agglutination (---▲---) in infected neonate.

Fig 2 Correlation between CRP concentration and slope of aggregometer tracing: $y = 22.0 \times + 8.6$; $r = 0.954$; $N = 27$.

Some take more than a day to perform while others require expensive equipment. The agglutination method is rapid, reproducible, inexpensive and easy to perform in a laboratory with a platelet aggre-gometer. Because single measurements of CRP concentrations give limited information this method could be used as a screening method in the serial monitoring of at risk patients—for example, those with leukaemia or those in the neonatal intensive care unit.

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Letters to the Editor

Spurious increase in plasma potassium concentration and reduction in plasma calcium due to in vitro contamination with liquid potassium edetic acid at phlebotomy

Recently an increased number of spuriously raised plasma potassium values were noted in the routine workload of our biochemistry laboratory, an incidence of about 1 in 1000 samples. Six months previously a blood tube (Sarstedt) containing liquid potassium edetic acid anticoagulant had been introduced by the haematology department. Obvious sources of plasma contamination, such as aged samples, haemolysis, sampling from intravenous lines, and batch contamination of the lithium heparin tubes used for plasma potassium determination were excluded. It seemed plausible that the increased potassium values could have been caused by droplet transfer of liquid potassium edetic acid anticoagulant from the Sarstedt tubes to lithium heparin tubes when the latter were being filled from a syringe after phlebotomy.

To test this hypothesis 30 Sarstedt tubes containing visible droplets of liquid potassium edetic acid at their apertures and 40 lithium heparin bottles from a single batch were selected. Ten millilitres of pooled heparinised blood was drawn up into each of 40

fresh syringes. Thirty test samples were prepared by injecting 2 ml of blood from each syringe into a blood tube containing liquid potassium edetic acid, with the syringe tip resting against the side of the tube, and then dispensing the remaining 8 ml of blood into a lithium heparin bottle. Pooled blood dispensed directly from 10 fresh syringes into 10 lithium heparin bottles served as controls.

Blood in the lithium heparin tubes was analysed for potassium using flame photometry and for calcium by the o-cresolphthalein complexone dye method, which detects only non-chelated calcium and thus gives a low plasma calcium value in the presence of edetic acid.

The table shows our results. Obvious differences were noted between control and test mean plasma potassium values and mean plasma calcium values measured by the dye method.

Spurious hyperkalaemia and hypo-

calcaemia can clearly arise due to in vitro contamination with liquid potassium edetic acid. This may therefore be an unsuitable form of anticoagulant for clinical practice unless strict precautions are taken to prevent cross contamination.

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Table Mean (SEM) plasma potassium and mean plasma calcium values in control and test plasma samples

Group	No of patient samples	Plasma potassium (mmol l ⁻¹)	Plasma calcium* (mmol l ⁻¹)
Control	10	4.65 (0.1)	2.10 (0.08)
Test	30	5.65 (0.59) range 4.5–7.2	1.65 (0.24) range 0.83–2.02

*Plasma calcium estimated using Technicon SMA12/60 dye method (o-cresolphthalein complexone)