Loctite UV 357 polymerised glass adhesive as a section mounting medium

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The permanent encapsulation of cell monolayers and histological sections on to microscope slides is usually performed using a plastic and solvent system such as XAM or DPX (British Drug Houses, Poole, Dorset UK). Because of the solubility of some of the coloured stain and reaction products increasingly used in newer enzyme substrate based staining techniques and the damage caused to sections from plastic embedded tissue by the hydrocarbon solvent in these preparations, it is not always possible to use this system. A further disadvantage of conventional mounting media is that until the solvent has evaporated, coverslip fixation is not permanent. Alternative mountants are available, but almost all have disadvantages which give them a more limited range of uses than conventional types of media. There is a need for a mounting media that can, in certain circumstances, be substituted for conventional mounting media. Such a medium should ideally have all the advantages but none of the drawbacks of the plastic and solvent system and be easy to use. In this paper the properties of one possible substitute were investigated.

Material and methods

Loctite UV 357 (Loctite (UK), Welwyn Garden City, Hertfordshire, UK) is one of a group of adhesives designed for bonding glass to glass. It is a low viscosity, fast curing, low toxicity, single component urethane resin specially designed for optional bonding applications. It is fully polymerised within 30 seconds by long wavelength ultraviolet light, ideally with a spectral energy distribution concentrated at 365 nm, and because of its clarity (transmittance >98%) when polymerised offers the potential of rapid high quality section encapsulation. Loctite 357 UV resin has been used here as a direct replacement for the plastic and solvent medium in mounting sections prepared and stained using a variety of techniques.

CONVENTIONAL LIGHT MICROSCOPY

Sections from paraffin wax embedded tissue or frozen sections were stained using conventional dye tech-

niques. Preparations were allowed to air dry after being rinsed in distilled water or taken to diethyl ether after dehydration in alcohol. One drop of Loctite 357 resin was then placed on to the section, the coverslip applied and firmly pressed down. At this stage the coverslip was still mobile and its position on the slide could be adjusted; it can, if necessary, be removed. A permanent mount was achieved by exposing the section to ultraviolet light from a lamp held in close proximity to the slide for about 30 seconds.

ENZYME HISTOCHEMISTRY

Cytochrome cell monolayers of synovial fluid cells and frozen sections of lymph nodes were reacted for non-specific esterase activity using α -naphthyl propionate with fast garnet GBC salt¹ as localising reagent. Preparations were rinsed in distilled water, allowed to air dry, and mounted in Loctite 357.

PLASTIC SECTIONS

Bone biopsy sections were stained "free floating" and therefore required mounting on to the microscope slide before applying the coverslip. Entrapment of air is a very common problem when sections are mounted like this, no matter what the mounting medium. Loctite 357 can be used to circumvent this problem in the following way. Firstly, the unstained 5 or 10 micron section was floated on to distilled water at 70°C in a Petri dish (the heat relaxes the resin and flattens the section), when cooled the flattened section was stained "free floating". Once stained, the section was suspended in water from where it was picked up on to a strip of 175 micron Melinex film (Imperial Chemical Industries PLC, The Heath, Runcorn, Cheshire, UK). This was achieved by sliding the end of the film under the surface of the water and with the aid of a fine pair of forceps the section manoeuvred on to the film, this then being dried on a hotplate at 70°C. Once dry the section adheres to the Melinex film. One drop of Loctite 357 was put on to the section and a cleaned slide pressed on to this and any bubbles gently squeezed out the slide, and the Melinex sandwich was then polymerised using ultraviolet light. When fully polymerised (about one minute), the Melinex film was peeled off (Melinex film does not adhere to polymerised Loctite resin), leaving the section adhered to the slide. One drop of Loctite 357 was then placed on to the section, the coverslip applied, and the resin polymerised.

Results

Conventional stained sections, when mounted with Loctite 357 resin, were not subject to fading or translocation of dye, when examined at bimonthly intervals over the course of two years, and because the

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.

References

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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-