Technical methods

3-Aminopropyltriethoxysilane (APES): a new advance in section adhesion

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Various adhesives have been used to increase the adherence of tissue sections to glass slides and to prevent separation during staining. Traditionally glycercin albumin and gelatin solutions have been widely used in routine histology. More recently poly-L-lysine has been used to improve section adhesion, particularly in immunocytochemistry. This method has been of great value but it is time consuming and slides cannot be stored.

For the simultaneous immunostaining of T6 and S100 in tissue sections, we have found it necessary to use frozen sections of tissue fixed in formol calcium. With poly-L-lysine used as the adhesive, there was a high loss of sections after initial protease digestion. An alternative method for increasing adhesion was therefore sought.

For over 20 years alkoxysilanes have been used in an industrial context as coupling agents in coating and dyeing a variety of materials. Weetall et al described their use as coupling agents for insolubilising enzymes on inert surfaces, emphasising that the strong covalent bonding effect between the aminoalkyl groups and aldehyde or ketone functions of a reactive surface would withstand repeated washings with a variety of inorganic solvents. More recently aminoalkylsilane has been used as an adhesive for enhancing chromosome spreading on glass slides, and for in situ hybridisation of frozen sections.

In this paper we describe a new modification to the coating method for hybridisation originally introduced by Rentrop et al. The modified method is simple, cheap, and can be used in the routine laboratory, whenever improved adhesion of sections is necessary.

Method

3-AMINOPROPYLETRIOXYSILANE (APES) COATING OF GLASS SLIDES
1 Soak slides in 10% Decon at room temperature overnight.

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other lengthy rigorous cytochemical techniques.

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References

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**Rapid method for stained renal biopsy specimens embedded in epoxy resin**

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Embedding small biopsy specimens in epoxy resin (by preparing 1.5 μm sections and staining them with a variety of techniques) enhances optical resolution and accuracy of histopathological diagnosis.

For many years, all our renal biopsy specimens, routinely embedded in paraffin wax, were stained with haematoxylin and eosin, periodic acid Schiff, Martius scarlet-blue and periodic acid-methenamine silver methods,¹ which we were anxious to retain after switching to plastic embedded specimens. Epoxy resin was found to be preferable to acrylic resin, mainly because it could be partially removed to allow for good staining. Some technical modifications were necessary as thin plastic sections require more intense staining. We found that some stains benefitted from being heated while others required their times extending; and phloxine replaced eosin as it gave similar but stronger results.²

Since 1979, all routine, non-urgent renal biopsy specimens in this department have been embedded in epoxy resin. Until now, this procedure has taken at least four days, because specimens had to be hand

processed and the resin required polymerisation overnight at 60°C. The drying (overnight at 35°C) and staining of sections were also time consuming, making the process unsuitable for urgent cases (patients with rapidly deteriorating renal function and most transplant biopsy specimens).

During the summer of 1986, an automatic “Lynx electron microscopy Tissue Processor” was purchased for all the various users of the electron microscopy suite of our department, the following method was devised to combine the benefits of plastic embedding with the availability of stained sections within 24 hours of biopsy.

**Methods**

After collection the specimen is processed overnight on the tissue processor with a sufficient delay in 10% buffered formalin to permit adequate fixation. For needle cores, the programme takes five hours and is as follows:

- 1 35% alcohol for 30 minutes
- 2 75% alcohol for 30 minutes
- 3 95% alcohol for 30 minutes
- 4 100% alcohol I for 30 minutes
- 5 100% alcohol II for 30 minutes
- 6 Propylene oxide I for 15 minutes
- 7 Propylene oxide II for 15 minutes
- 8 50:50 resin:propylene oxide for 60 minutes at 40°C
- 9 Neat resin for 60 minutes at 40°C.

When larger pieces of tissue are submitted (such as small open wedge biopsy specimens), processing times

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