Using LR White for Electron Microscopic Immunocytochemistry

L.R. White resin has five advantages which can be exploited for the localisation of antigens in sections of fixed and embedded tissue under the electron microscope.

1) It is a hydrophilic embedding agent which means that ultrathin sections allow the passage of aqueous solutions even at neutral pH as opposed to epoxides and polyesters, ultra-thin sections of which are much less permeable.
2) Its lipid solvency is apparently low for a plastic embedding agent and therefore membrane and cytosol structures can be observed under the electron microscope even when osmium has not been used to stabilise lipids. No low temperature methods are required although tissue structure is much improved by perfusion fixation methods.
3) It does not, where it has so far been tested, prevent the demonstration of antigens by immunochemical techniques. No 'etching' or protease digestion has so far been necessary.
4) It is beam-stable, standing up well to even quite low KVs, thus representing a considerable advance over the more commonly used methacrylates.
5) It tolerates rapid, partial dehydration, accepting tissue from 7fY% ethanol. Such tissue has an improved antigenicity over tissue which has been fully dehydrated.

FIXATION

To preserve antigenicity post-fixation in osmium tetroxide is best avoided but then a variety of possibilities is available depending on the requirements of the investigator. If tissue blocks are kept small (1-3 mm3) four to six hours fixation in freshly depolymerised and purified 4% paraformaldehyde in a 0.1 M phosphate (Sorensen's) buffer pH 7 .3, is recommended for the preservation of maximum tissue antigenicity. Tissue is then washed overnight in buffer solution. Picric acid, included with formaldehyde, will improve tissue structure slightly and Zamboni's fixative (Stefanini et al, 1967) which is 4% phosphate buffered paraformaldehyde with picric acid can be used. Glutaraldehyde, with its greater power to cross-link proteins, undoubtedly stabilises tissue structure to a greater degree than does formaldehyde, but in turn, tissue thus fixed demonstrates a reduced antigenicity. It is strongly recommended that vacuum-distilled, purified forms of monomeric or trimeric glutaraldehyde are used, to increase method reproducibility and avoid deleterious fixative impurities. Three to four hours in 1-2% glutaraldehyde in 0.1 M phosphate (Sorensen's) buffer pH 7.3, is perfectly adequate, remembering that the lower the concentration of glutaraldehyde the higher the antigenic yield. Tissue is then washed overnight in buffer.
The effects of the avoidance of osmium tetroxide can be partially compensated, without detriment to tissue antigenicity, by the inclusion of picric acid in the glutaraldehyde solution. The suggested solution is as follows: 50% purified glutaraldehyde -2ml, picric acid (sat. aq) - 15 ml, and 0.1 M phosphate (Sorensen's) buffer pH 7.3, - 83ml (BGPA) giving a 1 % glutaraldehyde, 0.2% picric acid solution. The percentage of glutaraldehyde and picric acid can be increased to give more stable ultrastructure but this may be at the expense of antigenicity. Two to four hours for the fixation of l-3mm3 blocks is recommended. More membrane structure is seen with this fixative than when glutaraldehyde is used alone, especially if it is perfused into animal tissue, when the molarity of the buffer should be decreased (0.05 M-0.08 M).
Further steps, such as post-osmication or 'block-staining' should not be carried out because they may reduce immunocytochemical sensitivity - either through reduced antigenicity or enhanced background.

DEHYDRATION

Tissue fixed in aldehyde alone, after buffer rinses, is dehydrated in a graded ethanol series. Tissue fixed in aldehyde/picric acid is placed directly into 70% ethanol where some of the picric acid can be washed out. Reducing the time in, and concentration of, ethanol used in dehydration will often increase antigenic yields. L.R. White will accept tissue from 70% ethanol so that after two washes of 30 -60 minutes each, blocks may be transferred into L.R. White. When osmium is avoided tissue shrinkage can be a problem and tissue taken from fixative to 7fY% ethanol and then straight into L. R. White may show shrinkage artefact. This can be lessened by introducing an intermediate step of diluted L.R. White which is 2: I L.R. White to 70% ethanol. Be careful not to carry over large amounts of 70% ethanol when transferring tissue.

INFILTRATION

Even though the tissue may be taken from 70% ethanol into L.R. White no special procedures are necessary. One change for an hour, followed by an overnight change (preferably on a 'rotamix') and then a final change the following morning before embedding, using gelatin capsules, is usually sufficient unless the blocks of tissue are particularly large (i.e. in excess of 3 mm3). As in the application sheet on L.R. White in routine electron microscopy, blocks may be stored in unpolymerised resin at 40C for weeks if necessary.

POLYMERISATION

Some special care must be taken to make sure that tissues undergoing embedding are not exposed to temperatures in excess of 55°C if antigenicity is not to be impaired. The 'cold-cure' procedure (see 'using L.R. White for E.M.'), therefore, even though the tissue is not osmicated, should not be used (the exothermic reaction may exceed 60°C). Instead an accurate oven or incubator set at 50°C is preferred and a 24-hour polymerisation time is given. Although anaerobic polymerisation is advised for Beem capsules, in fact L.R. White, when contained in gelatin capsules, fully filled and tightly capped, polymerises well in a 50°C oven without further precautions.
Trimming, cutting and use in the electron microscope are as for the applications sheet for electron microscopy.

STAINING

The choice of immunolocalisation technique is entirely up to the user, and PAP, hapten-anti hapten, avidin-biotin or gold-colloid methods may all be adaptable. Some useful publications to help with this choice are given in the references at the end of this application sheet.
Of course, from time to time, ultra-thin sections of tissues fixed, processed and embedded as described above should still be stained with uranyl acetate (15 mins) followed by lead citrate (3 mins) on the grids as for routine electron microscopy. The appearance of such tissues is different from that of post-osmicated tissue and it is important that the observer understands the material.
Immunostained sections can be counterstained with lead citrate but if peroxidase techniques are in use this may confuse the picture.

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