A SIMPLE METHOD FOR REMOVING THE RESIN FROM EPOXY-EMBEDDED TISSUE

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Epoxy resins were first used as embedding media for electron microscopy by Maaløe and Birch-Andersen (1) and Glauert and Glauert (2) and have since been employed extensively in Great Britain by Huxley (3) and Robertson (4). Recent improvements in processing and embedding techniques by Luft (5) and Finck (6) have led to their acceptance in many laboratories throughout the United States.

In correlated studies using electron and light microscopy it is usually necessary to remove the embedding material from the thick sections destined for conventional light microscopy before they yield optimal results in cellular detail and staining qualities. Both xylol and acetone are excellent and rapid solvents for methacrylate, and removal of this plastic and subsequent staining for light microscopy are simple procedures (Bencosme *et al.*, 7). Epoxy resins, however, are not soluble in standard organic solvents. Fisch and Hofmann (8) have studied the controlled chemical degradation into soluble components of epoxy resins cured with phthalic anhydride. Applying some of their findings we have developed a suitable solvent for use in tissue-resin systems.

2.5 gm. of metallic sodium are cut into cubes $\frac{1}{8}$ of an inch or less on a side and dropped piece by piece into 25 ml. of methyl alcohol in a hood or well ventilated area. It should be borne in mind that under conditions of high humidity sodium can ignite and result in serious accident. Thus reasonable care should be taken in carrying out this procedure quickly and with caution. Solution of the sodium to form sodium methoxide is more rapid at 50–60°C. and during the process the level of the solution is maintained at 25 ml. as the alcohol evaporates. When the sodium is completely dissolved an equal volume of benzene is added. If a phase boundary is present additional methyl alcohol can then be added until the resulting mixture is clear. This stock solution (~50 ml.) is stored in a dark bottle and used as a solvent for epoxy resins either at full strength or suitably diluted in a mixture of equal parts of methyl alcohol and benzene.

Coverslips with mounted sections are placed in the solvent according to the following schedule.

	Solvent	Time
"Ultra-thin" sections	Diluted $\frac{1}{3}$ with methyl alcohol/benzene mixture	30 seconds–1 minute
Sections <2 μ thick	Diluted $\frac{1}{3}$ with methyl alcohol/benzene mixture	1 to 3 minutes
Sections >2 μ thick	Undiluted	3 minutes

After the resin is removed, the coverslips are rinsed in methyl alcohol/benzene mixture followed by 2 changes of acetone and distilled water. It is important not to agitate the coverslips, particularly in the undiluted solvent, as a precipitate may form over the section which is difficult to rinse off in subsequent treatment. Sections may then be dried in air and stored, but better results are obtained if they are stained immediately by standard techniques. We have obtained excellent results with Bencosme's toluidine blue procedure (7).

This solvent has proved completely successful for Araldite 502,[1] Araldite M,[2] and Araldite 6005[3] epoxy resins using dodecenyl succinic anhydride as hardener. Sections from which the resin has been removed stain brilliantly, and structural details appear to be undamaged by the solvent action. The stock solution has been stored to date

[1] Ciba Products Corp., Kimberton, Pennsylvania.

[2] Aero Research Ltd., Duxford, Cambridge, England.

[3] Cargille and Sons, Little Henry, New Jersey.

for 6 months in our laboratory without any perceptible deterioration in performance.

SUMMARY

A mixture of sodium methoxide, benzene and methyl alcohol has been used to remove the resin from sections of epoxy-embedded tissues. Thick sections prepared in this way stain brilliantly and reveal excellent cellular detail in the light microscope. The solvent mixture can be stored indefinitely.

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