than 1μ but these are rather too thin for general study of marrow.

OTHER STAINING OR HISTOCHEMICAL PROCEDURES

The following have been used with success: haematoxylin and eosin, Perls's reaction for ferric iron, the periodic acid-Schiff reaction for carbohydrates, the alcian blue method for acid mucopolysaccharides, the Feulgen reaction for desoxyribonucleic acid, the periodic acid-hexamine silver method, Luxol fast blue for myelin, solochrome cyanine as a general stain, Heidenhain's iron haematoxylin method, and simple stains with basic dyes such as methylene blue and cresyl fast violet. Van Gieson's picrofuchsin and connective tissue stains of the Masson type do not work. Poor results were given by the methyl green-pyronin stain (Trevan and Shorrock) for nucleic acids. (Technical details for all these methods may be found in any current text of histological technique, for example, that of Drury and Wallington, 1967.)

TISSUES OTHER THAN BONE MARROW

The method has been applied with success to a number of tissues fixed in either glutaraldehyde or formalin, including spleen, lymph glands, kidney, liver, heart valves, tissues of the alimentary tract, and the retina. Blocks varying in size up to about 15×20 mm have been handled but it is advisable to keep the tissue slices 1-2 mm thick to ensure complete impregnation by the hydroxyethyl methacrylate.

Blocks of decalcified bone marrow have also been studied. Decalcification in a 5% solution of the di-potassium salt of diamino-ethane-tetra-acetic acid (EDTA), adjusted to pH 7 by the addition of 40% sodium hydroxide, gives good results. Decalcification may be hastened by warming to either 37° or 56°C without detriment to the tissue. Wash in running water overnight before embedding in hydroxyethyl methacrylate and stain with the Dominici procedure.

I should like to thank Dr P. Kidd and Dr F. Kurrein for their encouragement and Mr G. D. Holland for the photographs.

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Preparation of thin epoxy resin sections from thick sections of paraffin-embedded material

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The value of thin sections prepared from epoxy resin-embedded tissue for the routine histological examination of renal biopsies and other tissues was emphasized recently by Eastham and Essex (1969), thus confirming the use of thin sections for renal tissues as advocated earlier by Jones (1957a). However, most epoxy resin techniques, when compared with paraffin methods, possess several disadvantages. They include a slow embedding rate, considerable restriction in area and thickness of specimen that can be processed satisfactorily, orientation problems, irretrievability of specimen once embedded, and resistance of sections to routine histological or histochemical methods.

If, as seems likely, the thin section epoxy resin method is to be used as a routine diagnostic tool then it is vital that most of these disadvantages be overcome. It is hoped that the method described in this paper will help to meet this need.

Reagents

ARALDITE

- 10 ml epoxy resinCY 212 (Ciba)
- 10 ml hardenerHY 964 (Ciba)
- 0.5 ml acceleratorDY 064 (Ciba)
- 1.0 ml di-N-butyl phthalate (British Drug Houses)

Prepared according to the method of Glauert and Glauert (1958):

The epoxy resin, hardener, and di-N-butyl phthalate were mixed together in a 100-ml conical flask for five minutes, heated at 60°C for a further five minutes, and then accelerator was added. The whole was then mixed thoroughly for 10 minutes and allowed to debubble for 20 minutes at 60°C before use. The mixture minus accelerator has a shelf life of one month at 4°C .

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medium for routine 1-2 micron sectioning. *Stain Technol.*, 42, 119-123.

Ruddell, C. L. (1967b). Embedding media for 1-2 micron sectioning. 2. Hydroxyethyl methacrylate combined with 2-butoxyethanol. *Stain Technol.*, 42, 253-255.

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REPELCOTE

This is available from British Drug Houses.

PHARMACEUTICAL GELATIN CAPSULES

These capsules (Parke Davis and Company) should be size 00.

Method

Thick sections (20–40 μm) from paraffin-embedded material (formalin or formol-mercury fixed) were cut, floated on a water bath, mounted on albumenized Repelcoted 3 in. $\times$ 1 in. glass slides, dried flat at 37° to 50°C with section face uppermost, dewaxed in two changes of xylene, and embedded in Araldite.

EMBEDDING

Excess xylene was removed from around the sections and several drops of prewarmed (60°C) Araldite were added directly to the sections. The area for thin sectioning was selected with the aid of an ordinary light microscope (Zagury, Model, and Pappas, 1968) and the substage condenser was racked down to give sufficient contrast for this purpose. The rounded end of the longer portion of a pharmaceutical gelatin capsule was trimmed with a warmed scalpel blade and the other end placed over the selected area so that the capsule was perpendicular to the glass slide. A further 2 drops of prewarmed partially polymerized Araldite were added to the cylinder of gelatin and the preparation was incubated at 60°C for one and a half to two hours to effect a seal between the capsule and the glass slide. The rounded end of the other half of the gelatin capsule, containing weighted plasticine, was placed on the trimmed end of the embedded capsule to ensure that the latter retained its perpendicular position during this process. After incubation the weight was removed and the cylinder of gelatin was filled to the brim with Araldite and incubated at 60°C for a further 24 to 48 hours. This time schedule was reduced to two periods of 30 minutes at 80°C and 100°C respectively when urgent results were required (see also Estes and Apicella, 1969). The whole preparation was removed and allowed to cool at 4°C. At this stage the capsule was separated quite easily from the glass slide with the aid of a scalpel.

SECTIONS

The surface of the Araldite block, containing the flat embedded tissue section, was trimmed to the required area and thin sections (0.4 to 1.0 μm) were prepared on an A. F. Huxley pattern

Cambridge ultramicrotome with the aid of a glass knife. The sections were floated on distilled water, exposed briefly to chloroform vapour, placed in a drop of distilled water on a grease-free glass slide, and dried at 50° to 60°C from half to one hour.

STAINING

Pretreatment of Araldite sections with bromine vapour (Yensen, 1968) was performed in a fume cupboard. Several drops of bromine were added to a Coplin jar and the jar was sealed with a well fitting lid. Vapour was generated rapidly by gently shaking the jar. Sections were exposed for 15 to 30 seconds, washed in two changes of acetone, and rinsed in distilled water. Unlike the hydrogen peroxide method used by Aparicio and Marsden (1969) no difficulty was experienced in retaining sections on the slide during this process, nor was there any difficulty either in staining sections with silver techniques (Fig. 1).

The sections could now be stained with most of the standard histological or histochemical methods applicable to formalin or formol mercury-fixed paraffin-embedded material. The bromine treatment effectively removed mercuric artefact. Nuclear detail was enhanced in routine haematoxylin and eosin, periodic acid-Schiff (McManus, 1948), and trichrome methods (Lendrum, Fraser, Slidders, and Henderson, 1962) by using the

Fig. 1 *Membranous glomerulonephritis. Silver positive clubs are clearly shown. Postosmicated, formol-mercury fixed, paraffin-Araldite embedded section. Silver methenamine stain $\times$ 1,200.*

Fig. 2 Chronic pyelonephritis. Formol-mercury fixed, paraffin-Araldite embedded section. PAS $\times 120$.

iron haematoxylin method of Slidders (1969). Fluorescence methods for demonstrating amyloid (Burns, Pennock, and Stoward, 1967; Puchtler and Sweat, 1965; Vassar and Culling, 1959) should also be enhanced because autofluorescence is considerably quenched and the excitation rays required are best suited to thin sections. Postosmication of dewaxed or brominated sections improved the contrast between tissue structures (Fig. 1) when stained with the periodic acid silver methenamine method of Jones (1957b).

Most stained preparations were rinsed in distilled water, dried rapidly on a hot plate (60°C), dipped in two changes of xylene, and mounted in DPX.

The thick paraffin sections were blotted firmly, directly after mounting, with Whatman's no. 50 filter paper wetted with water in order to avoid any bubbling artifact being produced during the drying process.

Comment

The method described helps to bridge the gap between paraffin and epoxy resin techniques while retaining the major advantages of both. Adaptation of the flat cryostat section technique

of Zagury *et al* (1968) to paraffin sections facilitated the selection of the exact area required for thin sectioning, and the halogenation method of Yensen (1968) permitted the staining of epoxy resin sections with routine staining techniques, while routine fixatives such as formalin or formol mercury were employed. Although the embedding time in epoxy resin can be markedly reduced, the slower method is recommended for routine use. The thickness, area, and retrievability of specimens are limited only by the initial paraffin method employed. The method lends itself to serial section, retrospective, quantitative, and fluorescence studies, and under certain circumstances it may also be used for electron microscope work.

The method is particularly valuable for the study of renal biopsies and is used routinely for this purpose in this laboratory.

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