# A NEW MARAGLAS, D.E.R.® 732, EMBEDMENT FOR ELECTRON MICROSCOPY

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A new epoxy embedding mixture for biological material recently has been introduced by Freeman and Spurlock (1). This mixture consists of Maraglas 655, Cardolite NC-513, dibutyl phthalate, and the curing agent benzyldimethylamine (BDMA). Maraglas has certain advantages over other resins and epoxies, notably, wide range of miscibility, low viscosity, ease of sectioning, good staining qualities, beam stability, and little background granularity. Although improvements have been made in the original technique (2), certain problems remain. The Maraglas mixture penetrates tissue very slowly, makes the tissue quite brittle, and has variable polymerization properties.

In this paper the use and advantages of a new polyglycol type flexibilizer as a replacement for Cardolite NC-513 in a Maraglas 655 epoxy formulation will be presented.

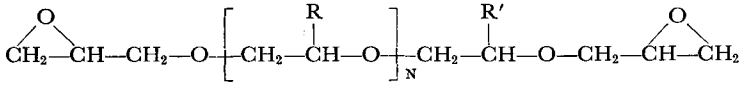
## MATERIALS

The embedding mixture contains the following materials:

1. Maraglas 655, Marblette Corporation, Long Island City, New York.
2. D.E.R.® 732, Dow Chemical Company, Midland, Michigan.
3. Dibutyl phthalate, Barrett Division, Allied Chemical and Dye Corporation, New York.
4. Benzyldimethylamine, Maume Chemical Company, Toledo, Ohio.

Maraglas 655 is a clear epoxy resin with a viscosity of 500 cps at 25°C in the uncured state. When fully cured it has a light transmission of 90 per cent and a heat distortion temperature of 87.8°C after 72 hours at 60°C. This temperature is high enough to reduce distortion to a minimum during electron bombardment. The resin is readily miscible with acetone and propylene oxide, but not with ethyl alcohol.

D.E.R. 732[1] is a polyglycol diepoxide flexible epoxy resin with the following theoretical structure:



$N \cong 5.6$

When used alone it gives soft cured compositions having low physical strength properties. The resin is a true epoxy and is compatible with practically all other epoxy resins. It also reduces the viscosity of epoxy formulations and is shelf stable after mixing. Since D.E.R. 732 is a true epoxy resin, it will react with all epoxy curing agents and become an integral part of a cured system. Its low viscosity (55–100 cps at 25°C), pale color (Gardner, max.—1), and low odor level make D.E.R. 732 quite desirable to use, since many other flexibilizing modifiers are dark in color, highly viscous, and have strong objectionable odors.

Dibutyl phthalate is an external plasticizer used to give hard but easily cut blocks suitable for sectioning. The tertiary amine BDMA, a catalytic curing agent that can be used in very small quantities, was found to be the most appropriate curing agent.

The following formulation was found to give the best results in this laboratory:

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|---|---|
| Maraglas 655 | 36 ml |
| D.E.R. 732 | 8 ml |
| Dibutyl phthalate | 5 ml |
| BDMA | 1 ml |

## FIXATION AND EMBEDDING PROCEDURE

Small pieces of solid tissue (or pellets of tissue culture cells) were fixed in phosphate-buffered (pH 7.4) 2 per cent osmium tetroxide containing glucose (osmolality—410 mOs/kg $H_2O$) for 1 hour (3).

The dehydration and infiltration procedure was as follows:—

| | |
|---|---|
| 50 per cent alcohol | 15 minutes |
| 70 per cent alcohol | 15 minutes |
| 95 per cent alcohol | 15 minutes |
| Absolute alcohol (2 changes) | 15 minutes |
| Propylene oxide (2 changes) | 15 minutes |
| Propylene oxide + epoxy mixture 1:1 | 45 minutes |
| Epoxy mixture (2 changes) | 1 hour and 2 to 3 hours |

Embed in polyethylene[2] (or gelatin) capsules, de-gas, and polymerize in 52°C oven for 17 hours. The tissue may also be left overnight in the epoxy mixture (at 4°C) before embedding, although this is not necessary.

Thin sections of tissue (silver to pale gold) were cut on a Porter-Blum microtome with either glass or diamond knives, and were expanded with xylene vapors. The expanded sections were then picked up on Formvar-carbon–coated grids and stained with uranyl acetate (4) and lead citrate (5). They were then examined in the Siemens Elmiskop I using the double condenser and a 50 $\mu$ aperture in the objective lens.

## RESULTS AND DISCUSSION

The resin hardened overnight into a crystal clear solid plastic. The tissue had the same consistency as the surrounding plastic; the blocks therefore could be trimmed and sectioned with ease. Any

[1] Dow Chemical Company Bulletin No. 170-144.

[2] BEEM capsules. Better Equipment for Electron Microscopy, Inc., Bronx, New York.

compression of sections could be relieved by exposure to xylene vapors. The sections were readily stained with uranium or lead salts (or both), and cellular preservation was excellent in a wide variety of tissue (Figs. 1–4). The sections were very stable in the electron beam, displayed little granularity at high magnification, and had a remarkably transparent appearance in the electron microscope.

The blocks turn yellow about one month after embedding. This is due to the oxidation of amino groups and possibly to other complex chemical reactions. This, however, does not seem to alter in any way the sectioning properties or cellular preservation. The tissue always appeared to be completely infiltrated, and bubble formation was hardly ever a problem in properly de-gassed blocks. Sections 1 $\mu$ in thickness could be cut and readily stained by a wide variety of routine procedures commonly used for light microscopy.

Certain precautions should be observed in the use of this embedment procedure. Freshly made up resin should be used, and it is necessary to leave the tissue in the resin at least 3½ to 4 hours before embedding. It is also advisable to use caution when handling the resin components, since allergic reactions may occur.

If the cutting properties of the basic mixture do not meet the individual requirements of the investigator, the mixture can be modified by varying the proportions of the D.E.R. 732 and Maraglas 655. In the 50 ml basic formulation, as little as 5 ml of D.E.R. 732 can be used to give a harder block, and as much as 15 ml to give a softer block. The amount of Maraglas 655 should be varied accordingly to maintain the original 50 ml volume. The omission of dibutyl phthalate will result in a slightly more brittle block. To avoid bubble formation, the capsule containing the unpolymerized epoxy and tissue should be de-gassed in a vacuum desiccator for at least ½ hour. Finally, it should be noted that the Maraglas 655 as it is obtained from the manufacturer may display some variability from can to can. If it is found that sections in the trough cannot be expanded by xylol vapors, then the Maraglas should be discarded and a new can should be tried. It is, therefore, desirable that blanks be run on each new can of Maraglas.

The results obtained in this and other laboratories seem to indicate that this new epoxy formulation gives consistently good results on a wide variety of biological material.

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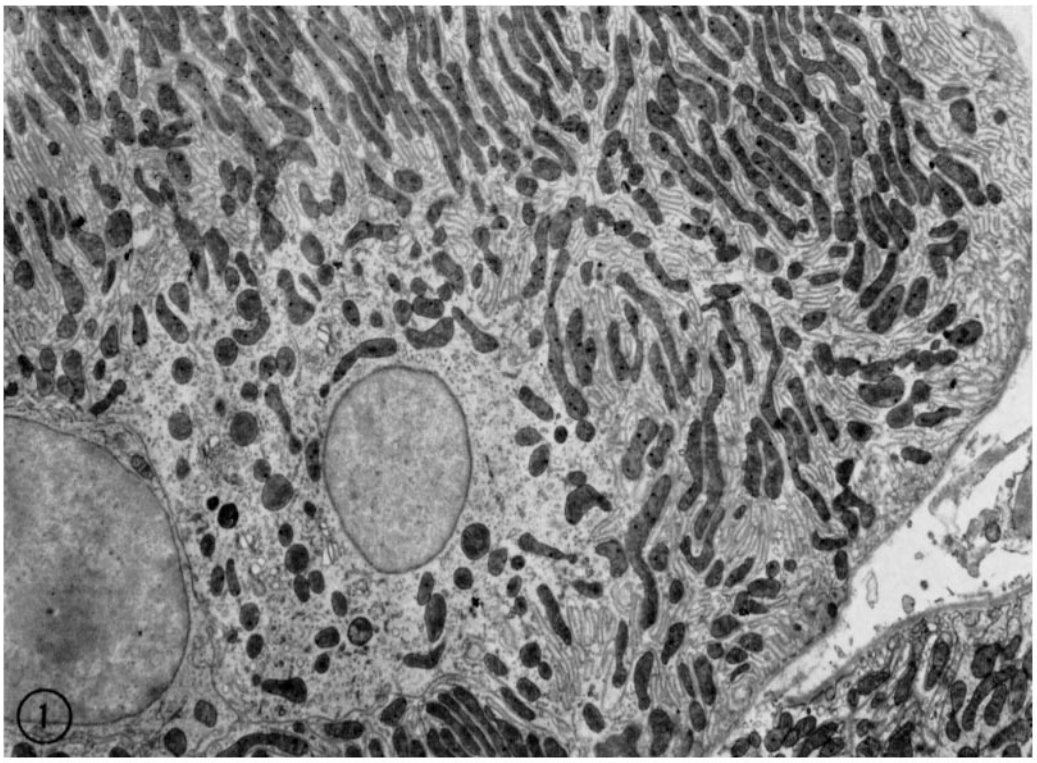
All sections have been double-stained with uranyl acetate and lead citrate.

FIGURE 1 A section through a portion of the convoluted tubule of mouse kidney, showing good preservation of membranes and cytoplasmic elements. × 5,700.

FIGURE 2 A section through the interlobular connective tissue of a rat pancreas. A portion of a duct is at the top of the picture. Nerves, capillaries, fibroblasts, and collagen can also be seen. × 2,800.



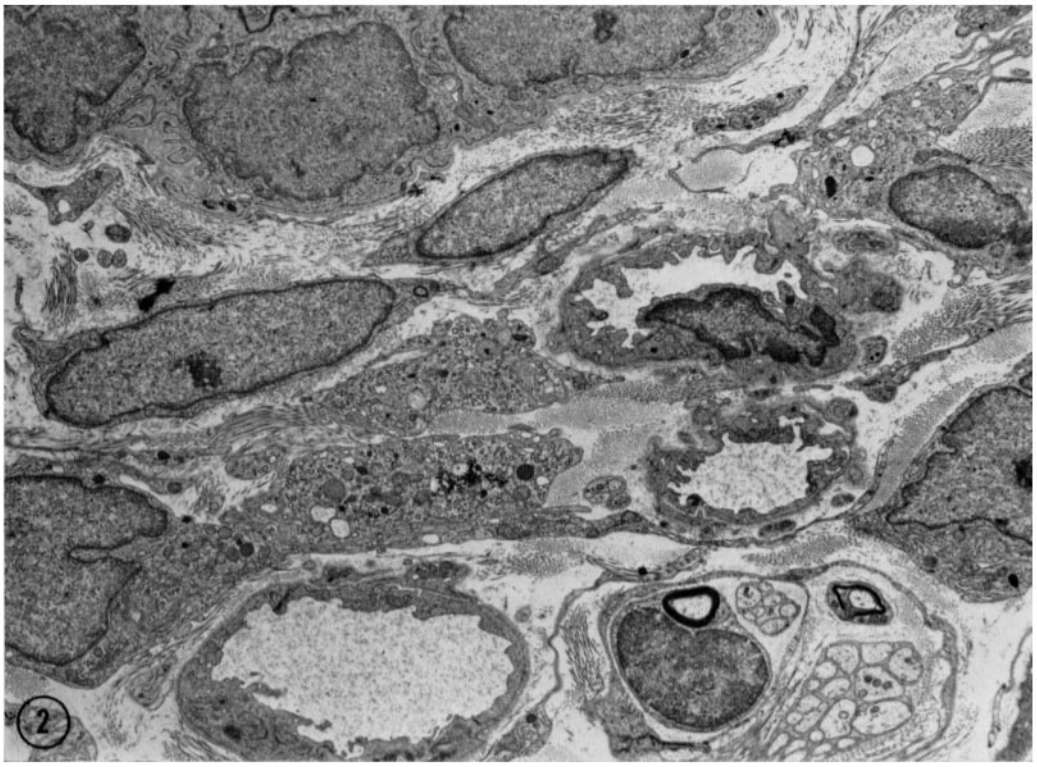


FIGURE 3 A portion of the cytoplasm of a mouse hepatic cell, showing excellent mitochondrial preservation. × 66,000.

FIGURE 4 Spontaneous mammary carcinoma of a C3H mouse, showing typical virus particles budding off the plasma membrane. Free virus particles measuring 120 mμ in diameter can be seen in the luminal space. × 100,000.