A NEW EPOXY EMBEDMENT FOR ELECTRON MICROSCOPY

JAMES A. FREEMAN, M.D., and BEN O. SPURLOCK

From the Department of Pathology, Louisiana State University School of Medicine, New Orleans

ABSTRACT

A new epoxy embedding mixture has been developed utilizing Maraglas 655 and Cardolite NC-513 with benzyldimethylamine (BDMA) as a curing agent. This epoxy mixture permits cellular preservation comparable to that obtained with Epon 812, ease of preparation of tissues, a wide range of miscibility, low viscosity, and, most important, ease of sectioning on a Porter-Blum microtome. In contrast to Epon-812–embedded tissues, Maraglas-Cardolite–embedded tissues can be sectioned in large dimensions with ease and consistent results without "chatter." No background granularity is detectable with high magnification study of Maraglas-Cardolite–embedded tissues. This epoxy is readily stained with lead hydroxide and is relatively stable in the electron beam.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023

Shortly after the introduction of methacrylate as a resin for embedding in biological electron microscopy (1), it became evident that significant undesirable polymerization damage was inadvertently incurred with its usage. In an effort to eliminate polymerization damage in embedments the epoxy resins were introduced in Denmark in 1956 by Maaløe and Birch-Andersen (2). This epoxy, Araldite, was improved by Glauert, Rogers, and Glauert in Great Britain and successfully employed by Birbeck and Mercer (3, 4). Difficulties arose in obtaining this epoxy from England for use in this country, and embedding with equivalent products marketed by the United States counterprart of the same manufacturer was only partially successful. When Kushida in Japan and Luft and Finck in the United States introduced Epon 812, a Shell Corporation product manufactured in the United States, it was widely adopted (5–7). Epon 812 exhibited the same excellent preservation of cellular fine structure as Araldite. Moreover Epon 812 sections yielded better contrast in the electron beam than did Araldite sections. The minimization of contrast problems was enhanced by the development of staining techniques (8, 9). Despite the advantages of Epon 812 many laboratories have been unable to utilize this epoxy resin routinely because of the "chatter" artifact produced in sectioning. The specimen area is necessarily small with trimmed Epon specimens, in contrast to the relatively large dimensions possible with methacrylate embedding. This creates a significant disadvantage for workers intent upon large-dimension sections used in other techniques such as radioautography. Also, the inherent granularity of Epon 812 may limit high magnification, high resolution studies.

These difficulties prompted the investigation of other epoxies. Excellence of preservation of cellular detail has been achieved in our laboratory with an epoxy mixture based on Maraglas 655.

MATERIALS

The following materials are utilized in this technique:

1. Maraglas 655—Marblette Corporation, Long Island City, New York.
2. Cardolite NC-513—Minnesota Mining and Manufacturing Company, Newark, New Jersey.
3. Benzyldimethylamine — Maume Chemical Company, Toledo, Ohio.
4. Dibutyl phthalate—Barrett Division, Allied

Chemical and Dye Corporation, New York City.

Maraglas 655[1] is a clear epoxy resin with a viscosity of 500 CPS at 25°C in the liquid, uncured state. It cures to a solid state having a heat distortion point of 190°F (87.8°C) after 72 hours at 60°C. It is readily miscible with acetone, propylene oxide, and styrene, but not with ethyl alcohol. The resin can be stored at room temperature (25°C) in glass-stoppered bottles for prolonged periods of time without increasing viscosity or partial curing. Maraglas 655, used alone, produces an extremely brittle, crystal clear embedment with a Rockwell hardness of 50 to 100 M. Hardness and brittleness can be varied utilizing long chain mono-epoxide resin flexibilizers and diluents such as Cardolite NC-513.

Cardolite NC-513[2] is an amber liquid resin with one epoxy group per molecule, therefore it combines chemically in any cured epoxy resin formulation. With a viscosity of 50 CPS it will reduce the viscosity of an epoxy formulation and improve penetrability of the liquid resin mixture. This mono-epoxide is compatible with the short chain non-reactive diluent propylene oxide used in the dehydration process. Varying the percentage of Cardolite NC-513 will vary the hardness as well as the brittleness of the embedment. Cardolite NC-513 is supplied by the manufacturer in metal containers. It is advisable to transfer the mono-epoxide to dark-colored, ground glass-stoppered bottles, to prevent accumulation of metallic particles in the liquid resin, which then can be stored at room temperature (25°C). There is a tendency for air accumulation and softening between the gelatin capsule and the embedment after prolonged storage of cured embedded specimens containing a concentration of Cardolite NC-513 greater than 30 per cent.

Any usual epoxy resin curing agent can be used with Maraglas 655 or Cardolite NC-513. Since it is more desirable to use minimum quantities of curing agent, the tertiary amine benzyldimethylamine (BDMA) was employed. The amount of this catalytic curing agent can be altered to regulate the speed of curing and, to some extent, the hardness and brittleness of the final embedment. BDMA can be employed in concentrations of 1 to 15 phr (parts per hundred of resin, by weight), the most desirable result being achieved with *ca.* 5 to 8 phr (*ca.* 2 per cent by volume) (10).

Dibutyl phthalate can be used in event the investigator wishes to vary the hardness, without increasing the brittleness of the specimen, for sectioning.

The following mixtures have been used:

Mixture	A	B	C	D	E
	(per cent)	*(per cent)*	*(per cent)*	*(per cent)*	*(per cent)*
Maraglas 655	60	60	65	65	70
Cardolite NC-513	40	30	30	20	20
Dibutyl phthalate	—	10	5	15	10

The curing agent for all 5 mixtures is 2 per cent BDMA. Mixture A, although the most plastic, has yielded satisfactory results in our laboratory. Mixture E is the hardest; consequently it is the most difficult to section. The addition of dibutyl phthalate increases the propensity to chatter during sectioning.

PREPARATION AND USE OF MARAGLAS-CARDOLITE EPOXY EMBEDMENT

A complete dehydration in graded alcohols and propylene oxide is achieved according to the following schedule: 50, 70, and 95 per cent, absolute ethyl alcohol, 2 changes of the latter—15 minutes each; ½ part propylene oxide and ½ part epoxy mixture—30 minutes. The tissue can then be placed in the epoxy mixture for 1 hour and placed in fresh epoxy in predried gelatin capsules, then cured in an oven at 60°C for 48 to 72 hours.

Very thorough mixing of the Maraglas, Cardolite, and BDMA is paramount. Since the viscosity of the liquid resin mixture is below 500 CPS, no problem is encountered in impregnation of tissue. Thorough impregnation is achieved by "bathing" the tissue for about 1 hour in the epoxy mixture. Although the degree of cross-linkage can be varied by the temperature of the curing cycle, good curing can be obtained with a cure of 48 to 72 hours at 60°C without differential temperature cycles. Complete cross-linkage, although desirable, is chemically impossible. Disintegration of the sections will occur in 20 per cent acetone if the cure is not optimum. Occasionally the epoxy

[1] Marblette Corporation technical bulletin.

[2] Minnesota Mining and Manufacturing Company technical bulletin.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023

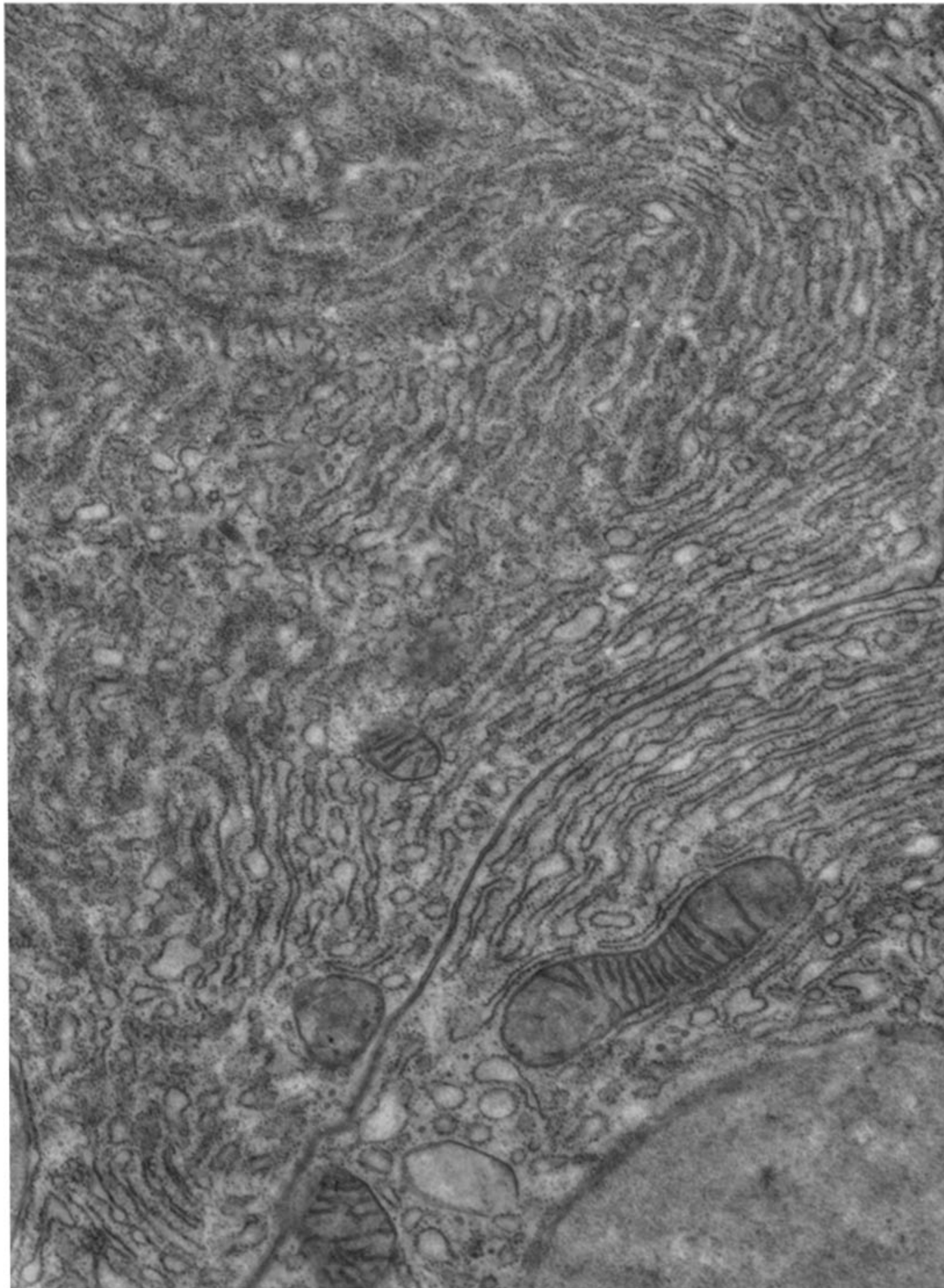

FIGURE 1

Portions of pancreatic acinar cells of the dog. The rough surfaced profiles of the endoplasmic reticulum are present. A portion of the nucleus is located at the lower right. Note the excellent preservation of structural relationships. Mixture A embedment. Lead hydroxide stain. × 19,000.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023

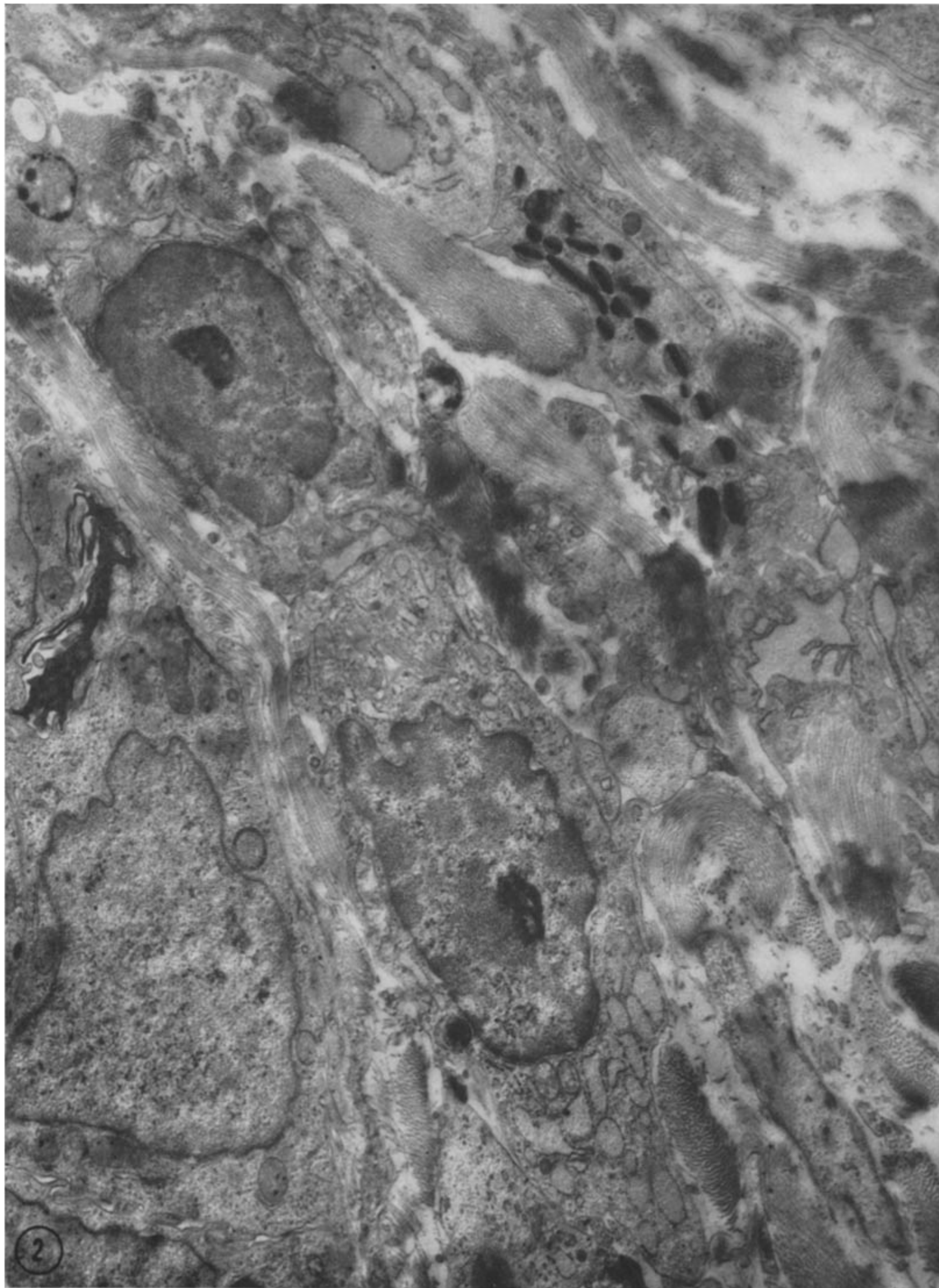

FIGURE 2

Lamina propria of the intestine of the rat. A basal epithelial cell is located at the lower left. Fibroblasts, a portion of an eosinophil, and collagen fibrils are closely packed. Irregular dense areas representing technical imperfections produced during sectioning can be seen in some of the collagen bundles in the center of the micrograph. Mixture A embedment. Lead hydroxide stain. × 12,000.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023

adjacent to the tissue may be somewhat soft. To remedy this problem, double embedding permits sectioning. To accomplish this double embedding, the tissue is cut out of the cured embedment, placed in a fresh gelatin capsule with fresh epoxy mixture and re-cured in an oven for 48 hours at 60°C. Any desired combination of Maraglas 655 and Cardolite NC-513 can be used; however, a mixture of Maraglas 80 per cent and Cardolite 20 per cent (2 per cent BDMA curing agent) has been found satisfactory. After double embedding in this mixture, the free plastic is brittle, but this does not alter the quality of the cellular preservation or the ease of sectioning. As is true of epoxies in general, the Maraglas-Cardolite mixture does not attain maximum hardness until permitted to remain at room temperature for 2 to 24 hours after curing and before sectioning.

The sectioned Maraglas-Cardolite embedments can be expanded with chloroform vapors. Routine gold sections can be obtained and expanded in the microtome boat, overcoming any compression artifact. Sectioned specimens can be readily stained with lead hydroxide by methods in current use (9).

An estimated 15 per cent over-all shrinkage occurs in stored blocks. The majority of shrinkage occurs at the gelatin-epoxy junction and no significant alteration of the specimen is detectable.

Sections of this embedment are relatively stable in the electron beam (50 and 100 kv) without membranes on 400-mesh grids or mounted on membranes on larger open mesh grids. We have found excellent stability with formvar membranes on Sjöstrand-type grids, enabling examination of large areas of the section.

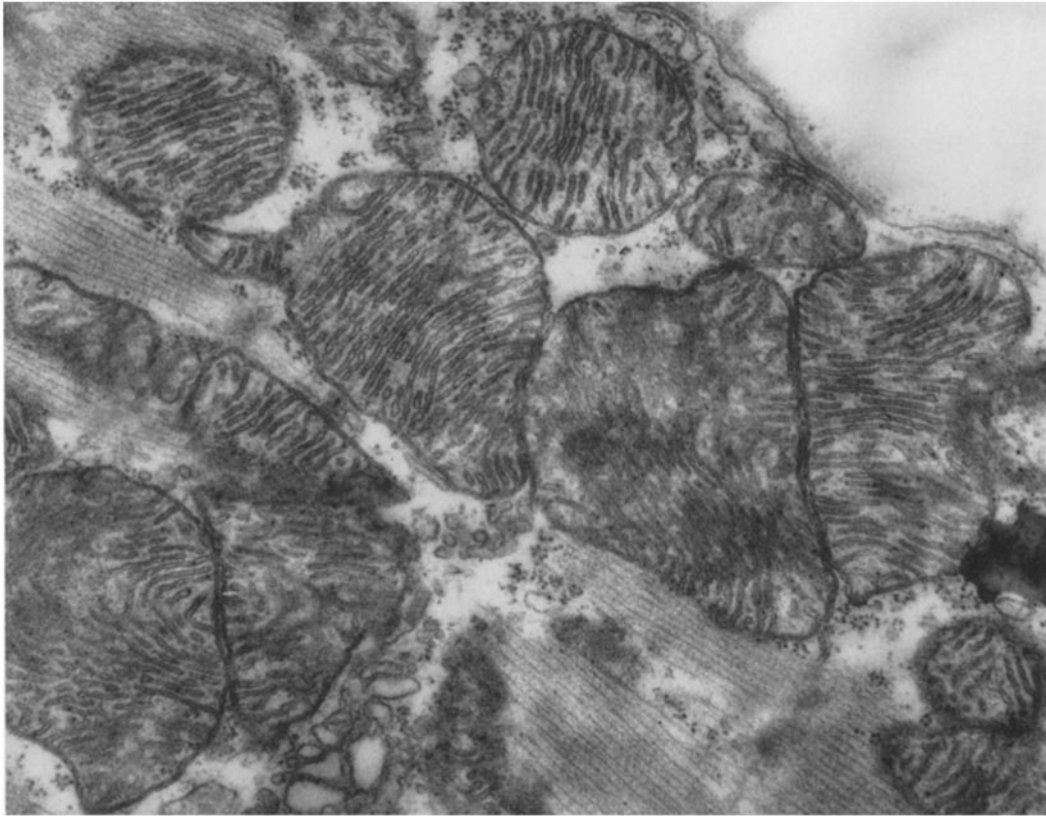

FIGURE 3
Mitochondria of cardiac muscle of the rat. The sarcoplasmic reticulum and glycogen are visible. The myofilaments are well preserved. Mixture A embedment. Lead hydroxide stain. × 38,000.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023

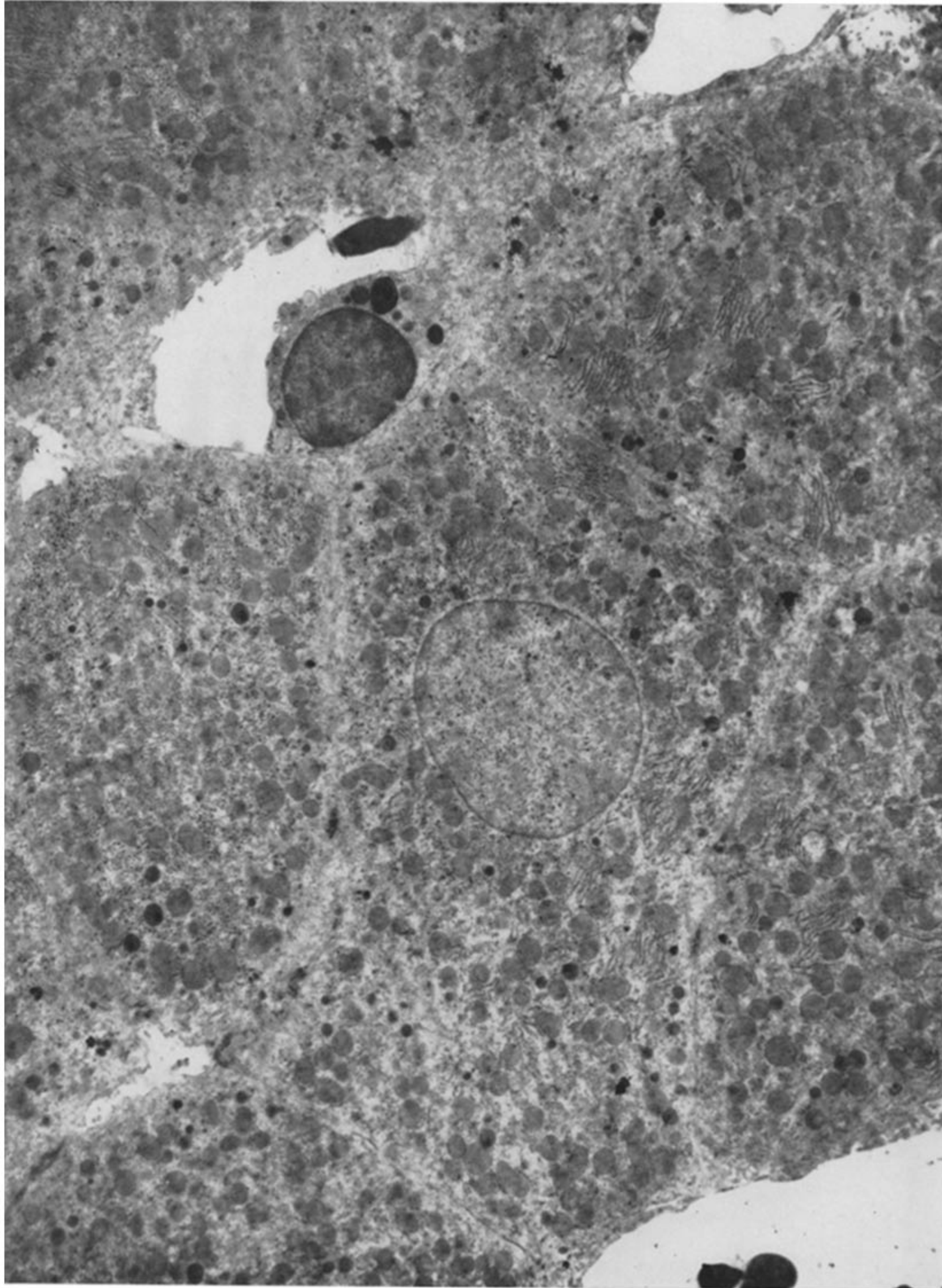

FIGURE 4
Portions of hepatic cord cells and sinusoids of the rat. This micrograph is from a section mounted without a membrane on a 400-mesh grid. The contrast of this epoxy is similar to that of other epoxies, *i.e.*, relatively low, but it can be photographically enhanced as is evident in this micrograph of an unstained section. Mixture C embedment. × 3,000.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023

The mixture can be stored, with curing agent added, in a freezer and thawed again before use. Constant usage of such a mixture, however, initiates a slow curing, thereby increasing the viscosity.

COMPARISON WITH EPON 812

Cellular preservation utilizing Maraglas-Cardolite epoxy mixtures is excellent (Figs. 1 to 4) and comparable to that achieved with Epon 812. The ease of preparation of tissues is greater than with Epon 812 and the low viscosity permits use at room temperature without heating the mixture. The reasonable ease of sectioning and the reproducible results with Maraglas-Cardolite epoxy are obvious desirable characteristics. In contrast to Epon 812 large areas can be trimmed and sectioned, thus making available broad areas of the specimen for viewing. These embedments are as stable in the electron beam as Epon 812. There is no noticeable background granularity of Maraglas-Cardolite specimens, thus permitting high magnification, high resolution work (Fig. 3); however, irregular dense areas representing technical imperfections are more frequent with Maraglas-Cardolite than with Epon 812 (Fig. 2). Although unstained sections have low contrast in the electron beam, the contrast is, nevertheless, sufficient for photography (Fig. 4).

Our thanks are due to Miss Catherine Catsulis for her technical assistance and valuable suggestions.
This work was supported in part by grants from the Louisiana Heart Association and the National Institutes of Health (H-2549).
Received for publication, January 30, 1962.

BIBLIOGRAPHY

1. NEWMAN, S., BORYSKO, E., and SWERDLOW, M., New sectioning techniques for light and electron microscopy, *Science*, 1949, **110,** (2846), 66.
2. MAALØE, O., and BIRCH-ANDERSEN, A., On the organization of nuclear material in *Salmonella typhimurium*, Bacterial Anatomy, 6th Symposium of the Society for General Microbiology, Cambridge University Press, 1956.
3. GLAUERT, A., ROGERS, G., and GLAUERT, R., A new embedding medium for electron microscopy, *Nature*, 1956, **178,** 803.
4. BIRBECK, M., and MERCER, E., Some applications of an epoxide embedding media (demonstration), 15th Annual Meeting of Electron Microscope Society of America, Boston, 1957.
5. KUSHIDA, H., On an epoxy resin embedding method for ultra-thin sectioning, *Electron-microscopy*, 1959, **8,** 72.
6. LUFT, J., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
7. FINCK, H., Epoxy resins in electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 27.
8. WATSON, M., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 475.
9. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 736.
10. LEE, H., and NEVILLE, K., Epoxy Resins. Their Application and Technology, New York, McGraw-Hill Book Company, 1957.

Downloaded from http://rupress.org/jcb/article-pdf/13/3/437/1479067/437.pdf by guest on 08 December 2023