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Embedding Resins: An Historical Perspective

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ABSTRACT

A brief history of tissue embedding as applied to transmission electron microscopy is given. Perhaps the first successful embedding matrix was a mixture of methyl and n-butyl methacrylate. This formulation penetrated tissues well and was easy to section with the glass knives available at the time, but tissue preservation was sometimes marginal. Methacrylates were followed by epoxy resins which gave much better tissue preservation although they were more difficult to section and did not penetrate tissue as well. Epoxy resins are today the dominant embedding resin. More recently, resins particularly suited for immunocytochemistry have become popular. The most notable of these are LR White and Lowicryl. Advances in freezing techniques have also made vitreous ice a viable embedding matrix for many applications.

KEYWORDS

Review, Tissue embedding, Transmission electron microscopy

INTRODUCTION

Four requisites for the biological applications of transmission electron microscopy were: (1) A suitable fixative for the preservation of cellular structure (osmium tetroxide [7], buffered osmium tetroxide [59], aldehyde [71,72]). (2) A matrix substance that could infiltrate biological material and encapsulate tissue components so that they could be thin sectioned and examined under an electron beam (naphthalene [7], celloidin/paraffin [60], methacrylate [56,57]). (3) A microtome that could thin section the tissue-matrix complex [7,60,64]. (4) Knives to cut the thin sections (glass [38], diamond [12]). All of these except for aldehyde fixation, came together in the late 1940's and early 1950's and are with us in approximately the same form today.

The primary interest in this discussion is item 2, the development of matrices for encapsulating biological material. Without such matrices, examination of tissue as we know it today, would not be possible.

Only the major steps in the evolution of tissue embedding are discussed in this report. These are presented more or less in chronological order with emphases on the methacrylates and epoxies, the two classes of resins used most extensively by the author. The primary interest is the introduction of new embedding matrices and not their subsequent development. Three books were particularly helpful in establishing the chronology of events [20,27,81], and some of the references in this report were taken from them. See TABLE 1 for a listing of the major classes of embedding matrices available to electron microscopists.

METHACRYLATES

The development of embedding resins was not a conscious event in which a product was formulated to meet the needs of the microscopist. Rather it was, as it is now, an adaptation of

TABLE 1: MAJOR CLASSES of embedding matrixes.

1949	methacrylate	n-butyl/methyl methacrylate (Newman et al)
1955; 1959; 1962	negative staining	PTA, uranyl acetate (Hall; Brenner and Horne; Valentine and Horne)
1956	epoxy	Araldite M (Glauert et al 1956)
1957, 1958; 1969	protein	gelatin (Fernández-Morán and Finean, Gilëv); serum/egg albumin (Farrant and McLean)
1958; 1960; 1962	polyester	Vestopal W (Ryter and Kellenberger a,b); Rigolac (Kushida); Selectron (Low and Clevenger)
1958, 1859; 1960	water-miscible epoxy	Aquon (Gibbons); Durcopan A (Stäubli)
1960; 1965	water-miscible methacrylate	glycol methacrylate (GMA; Rosenberg et al.); hydroxypropyl methacrylate (HPMA; Leduc and Holt)
1961; 1970	polymerized aldehyde	urea-formaldehyde (Casley-Smith), urea-glutaraldehyde (Peterson and Pease)
1973	vinyl polymer	polydialylphthalate (PDAP; De Mets)
1979 (approx.) to present	vitreous ice	see Plattner and Bachmann 1982, Menco 1986, Moor 1987, Sitte 1987, Dubochet et al. 1988
1980	removable (Wax)	polyethylene glycol (Wolosewick)
1980	acrylate-methacrylate	Lowicryl (Carlemalm et al.)
1982	acrylic	LR White (Newman et al.)
1983	Nanoplast	melamine-formaldehyde (Bachhuber and Frösch)
1979; 1985,1986	removable (Plastic)	Polystyrene (Frangioni and Borgioli); Plexiglas (Gorbsky and Borisy)

products generally available to the public through other channels. Thus it was that a common plastic, Plexiglas (or Lucite; i.e., methyl methacrylate), was adapted for the first truly successful embedding matrix. The adaptation consisted of mixing two methacrylates, methyl (which gave a very hard block) and n-butyl (which gave a very soft block) so that the resulting block was suitable for thin sectioning. The ratio generally settled on was approximately 2-parts methyl to 7-parts n-butyl methacrylate. Methacrylates were generally shipped with a small percentage of hydroquinone to prevent premature polymerization. The hydroquinone was sometimes removed before the methacrylates were used; but more often left as shipped since the resins could still be polymerized by adding more catalyst than might otherwise be used. Initially, these resins were most often catalyzed with benzoyl peroxide.

The resulting resin had some very desirable characteristics. Both methyl and n-butyl methacrylate have very low viscosity, and maintain this viscosity until polymerization is initiated (for days or weeks if one should wish). Thus, penetration of tissue was never an issue. Moreover, methacrylate blocks are very easy to section, which was an important aspect at the time since sectioning had to be done with glass knives. Image contrast in the microscope was also very good when compared to that of epoxy resins (compare Figs. 1A,B).

Although the methacrylates had many virtues, they were deficient in one very important aspect, namely, the spatial preservation of cellular ultrastructure. It was known, of course, that methacrylates underwent marked shrinkage (as much as 20% linear dimension) during polymerization and that this could account for some of the noted distortions. However, the real problem was more subtle; i.e., the tissue elements looked as though they had been displaced in a random fashion to leave micro areas of cytoplasm devoid of visible substance. A common term used to describe this phenomenon was "micro explosions." These explosions were occasionally tissue specific. Explosions were most often associated with the last stages of polymerization. For example, there were many occasions when I left an embedding that was already too viscous to flow, just to come in the next morning to see a specific tissue (in this case the initial cells of the maize root) blown into unrecognizable debris. In most cases, however, explosions occurred only on a microscale (e.g., compare Figs. 1A,B and Figs. 2A,B) and were not visible to the naked eye. Several methods for eliminating this problem were devised but none proved fully successful. Perhaps the most common method was to use partially polymerized resin for the last stages of infiltration to reduce the amount of heat generated as the resins polymerized (Note, polymerized methacrylates are soluble in their monomers if they are not cross linked) . This did not completely solve the problem although it sometimes seemed to help. Another problem was that methacrylate sections (whether cross linked or not) were very unstable in the beam. At least part of this instability was due to vaporization of a large percentage of the resin by the beam. This was one of the factors responsible for the good image contrast -- but it also contaminated the column which, in those days, was a never ending problem.[2]

Over the years there were many adaptations to the standard methacrylate formula; e.g., use of cross linking agents to improve stability and new catalysts to promote more even polymerization. There also were water-miscible methacrylates which were adapted for use in cytochemical studies and to prevent excess extraction of lipids (see TABLE 2). Mixtures of methacrylates and styrene [49] and other resins were also reported occasionally.

In retrospect, it might well be said that the most important contribution of the methacrylates was that they gave the first insight into cell ultrastructure and, quite often, excellent structural preservation. They marked the beginning of modern biological electron microscopy. Moreover, versions of them are still in use today as indicated in TABLE 2.

TABLE 2: Some common ACRYLIC-BASED embedding resin types listed in the order in which they were first reported.

1949	methyl and n-butyl methacrylate	Newman et al.
1958	polyester resin (Vestopal W)	Ryter and Kellenberger a,b
1960	glycol methacrylate (water miscible)	Rosenberg et al.
1965	hydroxypropyl methacrylate (water miscible)	Leduc and Holt
1980	Lowicryl	Carlemalm et al, Kellenberger et al.
1982	LR White	Newman et al.

NEGATIVE STAINING

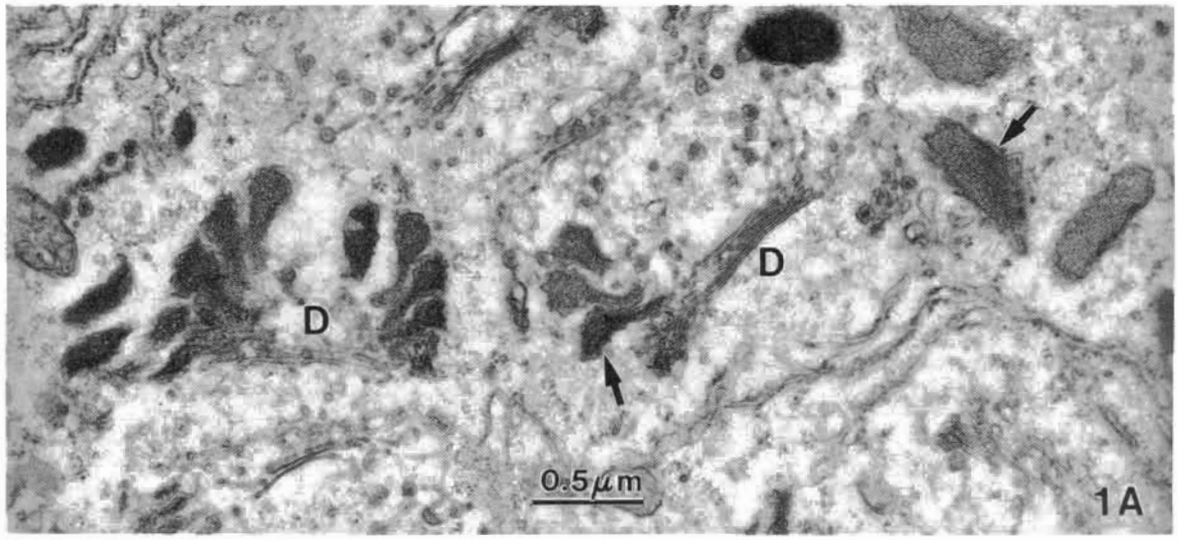
Not usually considered as an embedding matrix but indeed it is -- and a good one at that [4,10,26,28]. The tissue sample is encased in an amorphous layer of heavy metal that gives good image contrast with almost no sample shrinkage. Negative staining is capable of giving a specimen resolution of 0.5 - 0.8 nm which is far better than is possible with any other embedding matrix. The reason for this is that resolution is directly proportional to contrast (i.e., mass/density differences [81] and the embedding matrices generally used for negative staining are very dense. However, its use is limited to molecules and structures that can be spread thinly on a supporting membrane (Figs. 3A,B), or across small holes in a supporting film to give maximum resolution (not illustrated).

EPOXY RESINS

In 1956, the world of tissue embedding changed, perhaps forever, when epoxy resins were reported as suitable for tissue embedment [22,44]. These resins were easy to section, gave excellent tissue preservation (Fig. 1B), and had virtually no shrinkage. Some also were miscible with water [17,18]. Many formulations of epoxy resins have evolved over the years and several of these have become dominant embedding media

Figures 1A-C. Micrographs of glutaraldehyde/osmium tetroxide-fixed maize root cap outer cells. The tissues were fixed and dehydrated in the same way so that direct comparisons of contrast and tissue preservation could be made between the embedding matrices. [A] Tissue embedded in a 2:8 ratio of methyl methacrylate and n-butyl methacrylate respectively. Negative was printed on Agfa Rapitone GRADE P1 photographic paper. [B] Tissue embedded in an Araldite epoxy resin mixture. Negative was printed on GRADE P3 photographic paper. [C] Tissue embedded in Vestopal W. Negative was printed on GRADE P2 photographic paper.

Gross structural preservation of tissue elements is reasonably good in all of the preparations although contrast in the methacrylate embedded tissue is one or two grades of paper higher than that of tissues embedded in either epoxy or Vestopal resin. Note also that the background cytoplasm of the methacrylate-embedded tissue is pocketed with regions that appear devoid of substance. One other aspect is that images of methacrylate embedded tissues often appear slightly "soft" even when focusing has been adequate (also see Fig. 2A). Dictyosome or Golgi apparatus stack (D). Secretory vesicle (arrow).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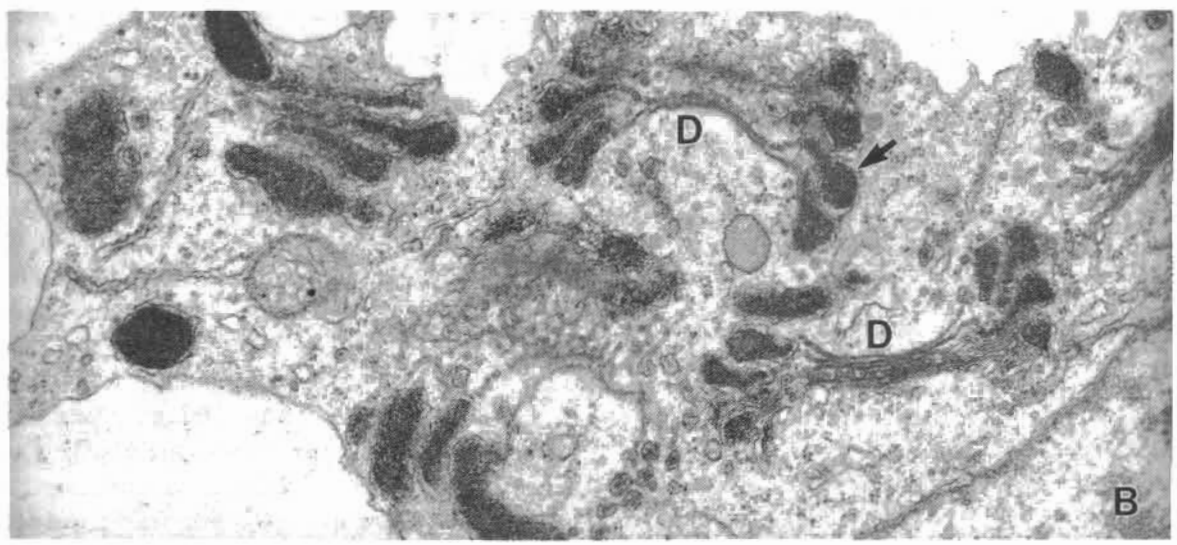
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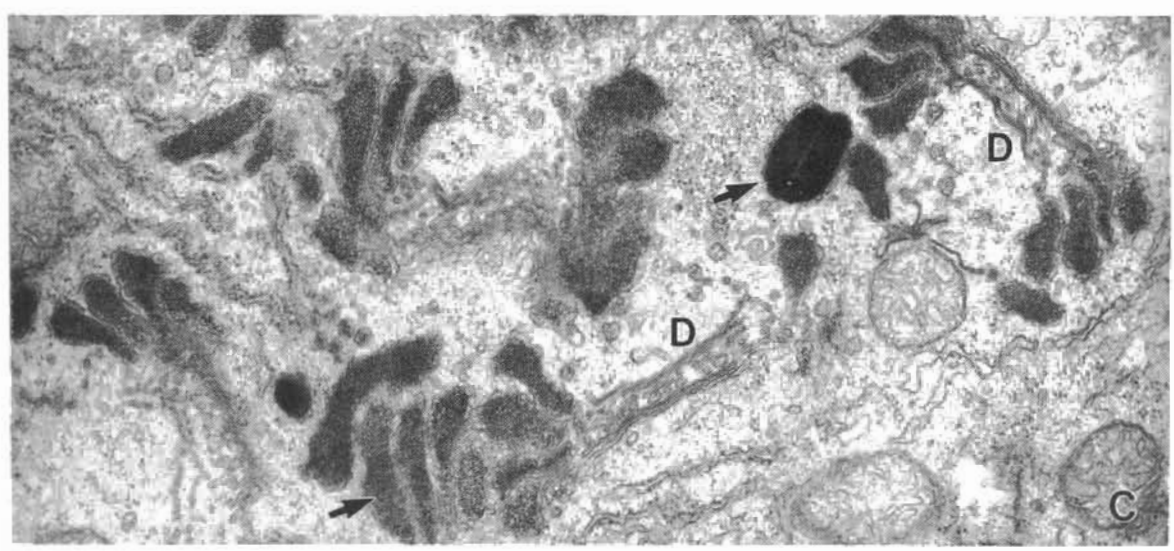
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where good structural preservation is required. However, epoxy resins are not without problems; e.g., all are probable mutagens [67] and one may be a carcinogen. They may be allergenic,[3] many are very viscous, and they react with some tissue elements which may limit their use for post-embedding cytochemistry [51-53]. Nonetheless, epoxy resins are the primary embedding resin in use today and probably will continue to be for many years to come, primarily because of their excellent spatial preservation of tissue elements. Many epoxy resin formulations have been introduced over the years; the major ones are listed in TABLE 3. TABLE 4 lists some properties of the most commonly used epoxy resins.

PROTEINS

Cross linked proteins were one of the early matrices for the preservation of ultrastructure (TABLE 5), their primary advantage being the retention of lipid components and other constituents extracted by non aqueous solvents. However, sectioning was usually a major problem since the blocks wet easily and, in most instances, had to be sectioned dry. Tissues also showed excessive shrinkage -- and the image was always in reverse contrast.

POLYESTER RESINS

Although never popular in the United States, these resins saw widespread use in Europe. These resins were cross-linked polymers of polybasic acids and polyhydric alcohols, or alkyd resins which gave very good tissue preservation with fairly low tissue shrinkage (Fig. 1C). Perhaps the most notable of these was Vestopal W [30,69,70]. However, the polyesters were all relatively viscous and not as easy to section as either methacrylates or epoxies, and they never gained the usage that might have been expected of them. The major polyester resins are listed in TABLE 6.

LR WHITE AND LOWICRYL

In spite of much progress, embedding matrices are still far from perfect and this has led to a continuing search for ever-better materials and embedding procedures. Of particular note are two resins that have found widespread use for post-embedment cytochemistry where the primary need is to preserve those molecules that are to be the recipients of a post-embedding marker. These are the acrylic and acrylate-methacrylate resins LR White [58] and Lowicryl [5], respectively. Both can be polymerized by heat, or by ultraviolet light at medium-to-very low temperatures, which aids in the preservation of protein structure and other labile molecules. Lowicryls can be obtained in either polar or non-polar forms. Preservation of tissue elements is often marginal and contrast may be very poor under the conditions often necessary for optimal cytochemistry. Neither is as stable under the beam as are the epoxy resins.

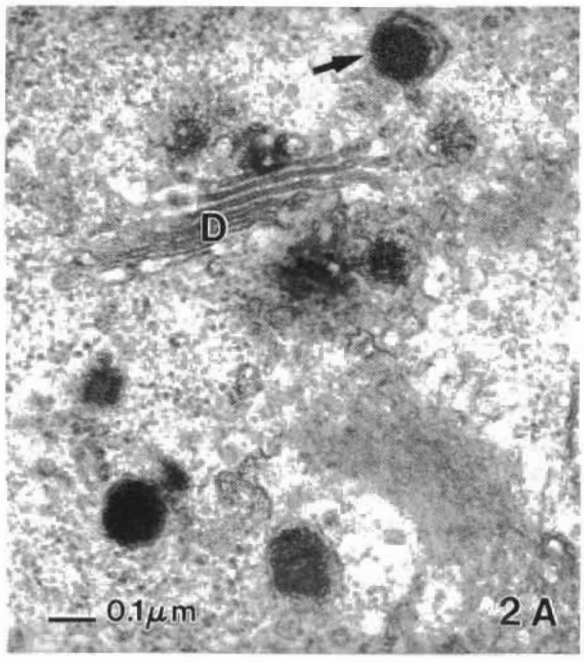
OTHER EMBEDMENTS

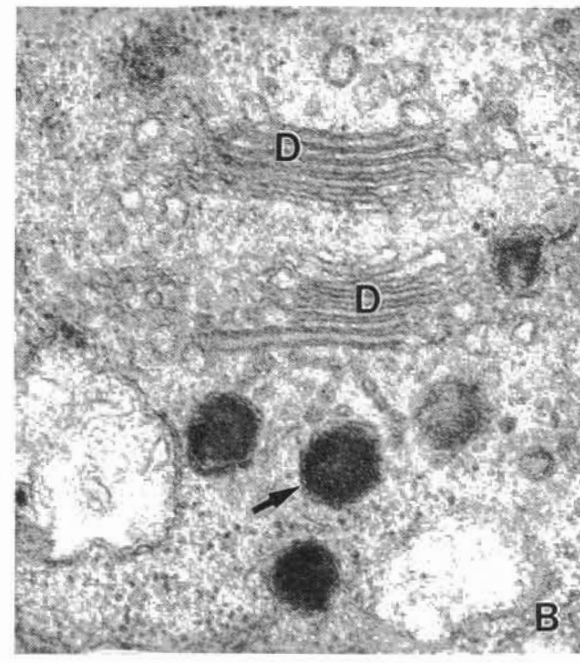
Many modifications to the embedding matrices listed above have appeared from time to time but most of these have not survived the years. It is interesting, however, that almost all of the major embedding matrices that have been developed and become popular are still available commercially today in approximately the same form in which they were originally formulated. Embedding matrices for special purposes have also appeared and three of these are noted: 1) A dense material for embedding to give negative contrast in section [1]. 2) Removable matrices (e.g., polyethylene glycol) for thick sections and high voltage microscopy [82]. 3) Melamine resins for ultra-high resolution [3]. Perhaps vitreous ice should also be considered as an embedding matrix since it, like other embedding media, retains cell structure so that tissues can be sectioned (or chemically fixed in subsequent steps) and then viewed in the electron microscope [29,48,55,66,77]. It offers the advantage of rapid embedding (milliseconds) without the use of non aqueous solvents. A partial listing of embedding matrices with major properties, is given in TABLE 7.

TABLE 3: Chronological list of the MAJOR EPOXY RESINS introduced over the years. Note that those investigators who published first were not always the ones who got major recognition for their efforts.

1956	Shell EPO	Maaløe and Birch Andersen
1956	Araldite M	Glauert et al, see Glauert 1991
1958, 1959	Aquon (mostly water miscible)	Gibbons
1958	Araldite CY 212 (Araldite M)	Glauert and Glauert
1959	Epon 812	Gibbons
1959	Epon 812, 815	Kushida
1960	Epon 812	Finck
1960	Araldite 502	Finck
1960	Durcopan A (water miscible)	Stäubli
1961	Araldite 502	Luft
1961	Epon 812	Luft (also see Luft 1973)
1962	Epon 812/Araldite 502	Voelz and Dworkin
1962	Maraglas 655	Freeman and Spurlock
1963	Epok 533 (QX 533)	Kushida
1964	Epon 812/Araldite 502	Mollenhauer
1964	Araldite 506	Mollenhauer
1964	DER 332	Lockwood
1964	DER 334	Winborn
1967	Epon 812/DER 736	Kushida
1969	ERL 4206 (VCD)	Spurr
1974	Quetol 651	Kushida
19--	Epon 812 substitutes	(Mascorro and Kirby 1986, 1987, 1990)

TABLE 4: CHARACTERISTICS of some of the more common EPOXY resin formulations.	
EPON 812	• Medium viscosity • Easy to vary block hardness • Polymerization begins very rapidly after mixing ingredients • Easy to section but prone to chatter • Good heat stability
ARALDITE M and ARALDITE 502	• Very high viscosity • Blocks usually very soft but can be moderated • Polymerization does not begin very rapidly after mixing ingredients • Lowest grain at high magnifications • Easy to section, no tendency to chatter • Moderate heat stability
EPON 812/ARALDITE 502	• Very high viscosity • Very soft blocks • Polymerization begins very quickly after mixing • Easiest of all epoxy resins to section, no tendency to chatter • Moderate heat stability
SPURR (Original Formulation)	• Very low viscosity (about 8 cps) • Very easy to adjust block hardness • Polymerization does not begin very rapidly after mixing ingredients • Easy to section and no tendency to chatter • Moderate to poor heat stability • Probable carcinogen, vapors harmful • Numerous spatial and staining artifacts • Contrast variable but usually fairly good
SPURR (Ladd adaptation using HXSA)	• Generally same as SPURR original formulation except: • Little or no spacial distortions • Very low contrast but can be improved by using • DER 736 in place of RC-1 (Mollenhauer adaptation) • Good heat stability





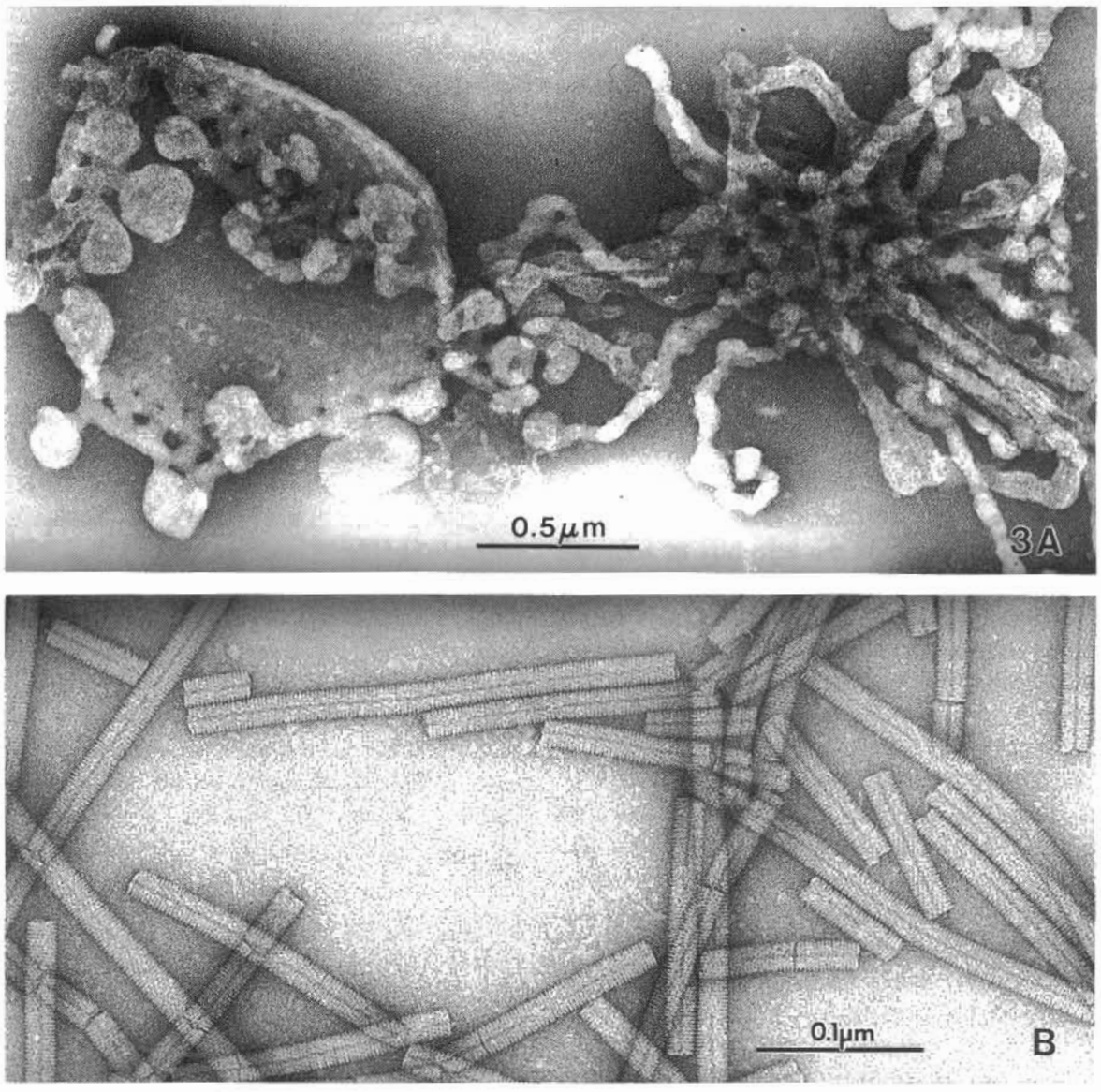
Figures 2A,B. Same as Figures 1A,B except that the cells are from the epidermis near the tip of the maize root, and the print magnifications are higher. Dictyosome (D) and secretory vesicle (arrow). Although both images define the structures being illustrated reasonably well, that of the epoxy-embedded tissue [B] is more pleasing than that of the methacrylate-embedded tissue [A] and is thought to be the more nearly correct. Figure B was printed on GRADE P3 paper and Figure A was printed on GRADE P1 paper.

TABLE 5: Chronological list of major PROTEIN embedding matrixes.		
1957	gelatin	Fernandez-Moran and Finean
1958	gelatin	Gilëv
1969; 1972	Serum, egg albumin	Farrant and McLean; Kuhlman and Viron

TABLE 6: Partial list of the most popular POLYESTER resins.		
1956; 1958	Vestopal W	Kellenberger et al., Ryter and Kellenberger (a,b)
1960	Rigolac	Kushida
1962	Selectron	Low and Clevenger
1964	Rhodester	Argagnon and Enjalbert
1972	Beetle	Rampley and Morris

TABLE 7: Partial list of the PROPERTIES and USE PATTERNS for several of the most common embedding matrixes (listed alphabetically).

Acrylate-methacrylate (e.g., Lowicryl)	Low viscosity; heat or low temperature light-activated polymerization; good for immunochemistry; polar and non polar formulations; semi-stable in beam. (e.g., Kellenberger et al. 1980, Carlemalm et al 1980)
Acrylic (e.g., LR White)	Hydrophilic; low viscosity; light or heat polymerization; fairly poor tissue preservation, good for immunochemistry. (e.g., Newman et al. 1982)
Epoxy	Medium-low to very high viscosity; low shrinkage; very good tissue preservation; easy to section; stable in beam; some are toxic. (e.g;., Glauert et al. 1956, TABLE 3)
Urea-aldehyde	Tissue impregnated with urea-formaldehyde and polymerized with ammonium chloride; Tissue impregnated with urea-glutaraldehyde and catalysed with oxalic acid; no lipid solvents or dehydration - good retention of lipids; poor tissue penetration; difficult to section. (Casley-Smith 1961; Peterson and Pease 1970, Pease and Peterson 1972)
Melamine (melamine/formaldehyde resin)	Hydrophilic; very hard; for very thin sections (8 nm+) and very high resolution; good tissue preservation. (e.g., Bachhuber and Frösch 1983)
Methacrylate	First practical embedding resin; often gave poor tissue preservation; high shrinkage during polymerization; unstable in beam. (e.g., Newman et al. 1949)
Metallic plastic (e.g., tin/styrene)	Dense supporting matrix; gives reverse image. (Acetarin et al. 1986)
Polyester (e.g., Vestopal)	Cross-linked embedding matrix; very viscous; stable in beam; good tissue preservation; not as easy to section as most epoxies. (TABLE 6)
Protein (e.g., gelatin, BSA, albumin)	Aqueous embedding; retains lipids; usually difficult to section. (TABLE 5)
Removable matrix substances (e.g., PEG; solubilized Plexiglas; vitreous ice)	May be removed from sections; good for thick sections/high voltage microscopy; immunocytochemistry; scanning EM. (e.g., Wolosewick 1980; Gorbsky and Borisy 1985, 1986; Sjöstrand 1951, Menco 1986)



Figures 3A,B. [A] Two cisternae isolated from a plant dictyosome, layered on a Formvar/carbon supporting film, and embedded in phosphotungstic acid. The cisterna on the left is from the midregion of a dictyosome and the cisterna on the right is probably from the cis pole of a dictyosome [54]. The dense parts of the image come from the embedding matrix rather than from tissue elements, and is the reason that this procedure is called negative staining. A resolution of less than 1.0 nm is easily achieved with this embedding method. [B] Micrograph of a plant virus showing the excellent resolution that can be achieved with negative staining. Micrograph from Dr. O. E. Bradfute, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio. x230,000.

EPILOG

And so the search for embedding matrices continues -- but not at a very fast pace. What is available now is acceptable for the most part although not ideal. Moreover, research support for such endeavors is seldom available. Nonetheless, progress is to be expected over the years to come, particularly in respect to ease of use, good penetration, good tissue preservation, special use resins, safety, and a better understanding of embedding itself. However, there are several problems that will be nearly impossible to surmount and will likely be with us for some time to come, most notably perhaps, sample/resin degradation in the beam. However, what is important is the recognition of how far the current procedures and the ingenuity of scientists and technicians have brought us. There is very little that we do not now know about the spatial relationships of most cellular constituents greater than 0.1 nm in size -- a rather remarkable achievement in only 40+ years.

ABBREVIATIONS

BSA	bovine serum albumin
DER	Dow Epoxy Resin
EM	electron microscopy
GMA	glycol methacrylate
HXSA	hexenylsuccinyl anhydride
PEG	polyethylene glycol
PTA	phosphotungstic acid

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[2]The most commonly available microscopes of this period were the RCA's EMU-2 and 3, and these had no interlocks for changing plates (3 or 5 exposure cassettes) or specimens. They also used silicon diffusion pump oil which back-streamed at a high rate. Apertures (platinum) were good for only a few hours and major cleaning of lenses and specimen holders occurred every week. Apertures often had to be cleaned with hydrofluoric acid to dissolve the glass-like coatings that formed on them from the beam/diffusion oil interaction.

[3]A technician of mine became so allergic to epoxy resins (in any form) that she could not enter a laboratory in which a bottle of resin was open. She ultimately had to leave electron microscopy for employment where epoxy resins were not used.

RESUMEN

Este trabajo presenta una breve historia de la aplicación del embebimiento de tejidos en la microscopia electrónica de transmisión.. Quizás la primera matriz de embebimiento exitosa fue una mezcla de metil y n-butil metacrilato. Esta formulación penetraba adecuadamente los tejidos y era fácil de cortar con la cuchillas de vidrio utilizadas para aquel tiempo, sin embargo, en algunos casos la preservación del tejido era limitada. Los metacrilatos fueron substituidos por las resinas epóxicas, con las cuales se obtuvo una mejor preservación del tejido, aunque presentaban mayores dificultades en la penetración y el seccionamiento del material. Hoy en día, las resinas epóxicas son el medio de inclusión más utilizado en microscopía electrónica. Recientemente resinas adaptadas para el uso en inmunocitoquimica se han convertido en medios de inclusión muy populares. Las más notables de estas resinas son LR White y Lowicryl. Los más recientes avances en métodos de congelación han hecho posible la obtención del "hielo vitreo", el cual, se ha convertido en un medio de inclusión viable para muchas aplicaciones.

REFERENCES

1. Acetarin, J.-D, Villiger, W., Carlemalm, E. (1986) A new heavy-metal-containing resin for low-temperature embedding and imaging of unstained sections of biological material. J. Electron Microsc. Tech. **4:** 257-264.

2. Argagnon, J., Enjalbert, L. (1964) Technique d'inclusion pour la microscopie électronique utilisant un polyester: le Rhodester 1108 CPSL. J. Microscopie **3:** 339-342.

3. Bachhuber, K., Frösch, D. (1983) Melamine-resins, a new class of water-soluble embedding media for electron microscopy. J. Microsc. **130**: 1-9.

4. Brenner, S., Horne, R.W. (1959) A negative staining method for high resolution electron microscopy of viruses. Biochem. Biophys. Acta **34**: 103-110.

5. Carlemalm, E., Villiger, W., Acetarin, J.D. (1980) Advances in specimen preparation for electron microscopy. I. Novel low-temperature embedding resins and reformulated Vestopal. Experientia **36**:740.

6. Casley-Smith, J.R. (1961) The preservation of lipids for electron microscopy; urea-formaldehyde as an embedding medium. Med. Res. **1**: 59.

7. Claude, A. (1948) Studies on cells: morphology, chemical constitution, and distribution of biochemical functions. Harvey Lect. **43**: 121-165.

8. De Mets, M. (1973) Studies on embedding materials. I. Polydiallylphthalate (PDAP), a new embedding medium for biological tissues. J. Ultrastruct. Res. **42**: 337-341.

9. Dubochet, J., Adrian, M., Chang, J.-J., Homo, J.-Cl., Lepault, J., McDowall, A.W., Schultz, P. (1988) Cryo-electron microscopy of vitrified specimens. Q. Rev. Biophys., **21**: 129-228.

10. Fabergé, A.C. (1974) Methylamine tungstate, a new negative stain. J. de Microscopie **20**: 241-246.

11. Farrant, J.L., McLean, J.D. (1969) Albumins as embedding media for electron microscopy. Proc. 27th Ann. Meet. Electron Microsc. Soc. Am., p. 422. Claitor's Publ. Division, Baton Rouge.

12. Fernández-Morán, H. (1953) A diamond knife for ultrathin sectioning. Exptl. Cell Res. **5:** 255-256.

13. Fernández-Morán, H. Finean, J. B. (1957) Electron microscope and low-angle x-ray diffraction studies of the nerve myelin sheath. J. Biophys. Biochem. Cytol. **3**:725-748 + Plates 229-240.

14. Finck, H. (1960) Epoxy Resins in electron microscopy. J. Biophys. Biochem. Cytol. **7**: 27-30.

15. Frangioni, G., Borgioli, G. (1979) Polystyrene embedding: a new method for light and electron microscopy. Stain Tech. **54**: 167.

16. Freeman, J.A., Spurlock, B.O. (1962) A new epoxy embedment for electron microscopy. J. Cell Biol. **13**: 437-443.

17. Gibbons, I.R. (1958) A water-miscible embedding resin for electron microscopy. Proc. 4th Internat. Cong. Electron Microsc., (Berlin) **2**:55, Springer-Verlag, Berlin.

18. Gibbons, I.R. (1959) An embedding resin miscible with water for electron microscopy. Nature, **184**: 375-376.

19. Gilëv, V.P. (1958) The use of gelatin for embedding biological specimens in preparation of ultrathin sections for electron microscopy. J. Ultrastruct. Res. **1**: 349-358.

20. Glauert, A.M. (1975) Embedding. In: Fixation, Dehydration and Embedding of Biological Specimens. Practical Methods in Electron Microscopy (A. Glauert, ed.), pp. 123-176, North-Holland Publishing Company.

21. Glauert, A.M. (1991) Epoxy resins: an update on their selection and use. Microscopy and Analysis, September 1991: 15-20.

22. Glauert, A.M., Rogers, G.E., Glauert, R.H. (1956) A new embedding medium for electron microscopy. Nature **178**: 803.

23. Glauert, A.M, Glauert, R.H. (1958) Araldite as an embedding medium for electron microscopy. J. Biophys. Biochem. Cytol. **4**: 191-195.

24. Gorbsky, G., Borisy, G.G. (1985) Reversible embedment cytochemistry. Proc. Natl. Acad. Sci. USA **82**:6889-6893.

25. Gorbsky, G., Borisy, G.G. (1986) Reversible embedment cytochemistry (REC): a versatile

method for the ultrastructural analysis and affinity labeling of tissue sections. J. Histochem. Cytochem. **34:** 177-188.

26. Hall, C.E. (1955) Electron densitometry of stained virus particles. J. Biophys. Biochem. Cytol. **1:** 1-15.

27. Hayat, M.A. (1970) Principles and Techniques of Electron Microscopy, Vol. 1, Biological Applications. Van Nostrand Reinhold Company, New York.

28. Huxley, H.E. (1956) In: First European Conf. Electron Microsc., Stockholm, p.260.

29. Kellenberger, E. (1990) The potential of cryofixation and freeze substitution: observations and theoretical considerations. J. Microsc. **161**: 183-203.

30. Kellenberger, E., Schwab, W., Ryter, A. (1956) L'utilisation d'un copolymère du groupe des polyesters comme matériel d'inclusion en ultramicrotomie. Experientia **12**: 421-422.

31. Kellenberger, E., Carlemalm, E., Villiger, W., Roth, J., Garavito, R.M. (1980) Low denaturation embedding for electron microscopy of thin sections. Chemische Werke Lowi, G.m.b.H., Waldkraiburg, FRG.

32. Kuhlmann, W.D., Viron, A. (1972) Cross-linked albumin as supporting matrix in ultrathin cryo microtomy. J. Ultrastruct. Res. **41:** 385-394.

33. Kushida, H. (1959) On an epoxy resin embedding method for ultrathin sectioning. J. Electron Microsc. **8**: 72-75.

34. Kushida, H. (1960) A new polyester embedding method for ultrathin sectioning. J. Electron Microsc. **9:** 113.

35. Kushida, H. (1963) An improved epoxy resin "Epok 533", and polyethylene glycol 200 as a dehydrating agent. J. Electron Microsc. **12**: 167-174.

36. Kushida, H. (1967) A new embedding method employing DER 736 and Epon 812. J. Electron Microsc. **16**:278.

37. Kushida, H. (1974) A new method for embedding with a low viscosity epoxy resin "Quetol 651." J. Electron Microsc. **23**: 197.

38. Latta, H., Hartmann, J.F. (1950) Use of a glass edge in thin sectioning for electron micoscopy. Proc. Soc. Exptl. Biol. Med. **74**: 436-439.

39. Leduc, E. and Holt, S.J. (1965) Hydroxypropyl methacrylate, a new water-miscible embedding medium for electron microscopy. J. Cell Biol. **26**: 137.

40. Lockwood, W.R. (1964) A reliable and easily sectioned epoxy embedding medium. Anat. Rec. **150**: 129-139.

41. Low, F.N., Clevenger, M.R. (1962) Polyester-methacrylate embedments for electron microscopy. J. Cell Biol. **12:** 615-621.

42. Luft, J.H. (1961) Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**: 409-414.

43. Luft, J.H. (1973) Embedding media - old and new. In: Advanced Techniques in Biological Electron Microscopy (J.K. Koehler, ed.), pp. 1-34, Springer-Verlag, Berlin.

44. Maaløe, O., Birch-Andersen, A. (1956) On the organization of the "nuclear material" in *Salmonella typhimurium*: In: Bacterial Anatomy (E.T.E. Spooner and B.A.D. Stocker, eds.), 6th Symp. Soc. Gen. Microbiol. University Press, Cambridge, pp. 261-278.

45. Mascorro, J.A. and Kirby, G.S. (1986) Physical characteristics of "old" Epon 812 and various Epon-like replacements. Proc. Electron Microsc. Soc. Am. **44**: 222-223.

46. Mascorro, J.A. and Kirby, G.S. (1987)

Eponate 12, a new epoxy resin: comparisons with Epon 812 and observations on its use for general biological electron microscopy. Proc. Electron Microscopy Soc. Am. **45**: 628-629.

47. Mascorro, J.A. and Kirby, G.S. (1990) Low viscosity, intermediate viscosity, and high viscosity alternatives in the family of epoxy resin embedding media --- your choice. Proc.Electron Microsc. Am., XII Internat. Conf. Electron Microsc. **3:** 138-139.

48. Menco, B.Ph.M. (1986) A survey of ultra-rapid cryofixation methods with particular emphasis on applications to freeze-fracturing, freeze-etching, and freeze-substitution. J. Electron Microsc. Tech. **4:** 177-240.

49. Mohr, W.P., Cocking, E.C. (1968) A method of preparing highly vacuolated, senescent, or damaged plant tissue for ultrastructural study. J. Ultrastruct. Res. **21:** 171-181.

50. Mollenhauer, H.H. (1964) Plastic embedding mixtures for use in electron microscopy. Stain Tech. **39:** 111-114.

51. Mollenhauer, H.H. (1988) Artifacts caused by dehydration and epoxy embedding in transmission electron microscopy. In: (Crang, R.F.E. and Klomparens, K.L., eds.), Artifacts in Biological Electron Microscopy. Plenum Publishing Corp., New York, pp. 43-64.

52. Mollenhauer, H.H. (1993) Artifacts caused by dehydration and embedding in transmission electron microscopy. Microsc. Res. Tech. (In Press)

53. Mollenhauer, H.H., Droleskey, R.E. (1985) Some characteristics of epoxy embedding resins and how they affect contrast, cell organelle size, and block shrinkage. J. Electron Microsc. Tech. **2:** 557-562.

54. Mollenhauer, H.H., Morré, D.J. (1991) Perspectives on Golgi apparatus form and function. J. Electron Microsc. Tech. **17:** 2-14.

55. Moor, H. (1987) Theory and practice of high pressure freezing. In: Cryotechniques in Biological Electron Microscopy, (R.A. Steinbrecht and K. Zierold, eds.), pp. 175-191, Springer-Verlag, Berlin.

56. Newman, S.B., Borysko, E., Swerdlow, M. (1949a) Ultra-microtomy by a new method. J. Res. Nat. Bur. Stand. **43**: 183-199.

57. Newman, S.B., Borysko, E., Swerdlow, M. (1949b) New sectioning techniques for light and electron microscopy, Science **110:** 66.

58. Newman, G.R., Jasani, B., Williams, E.D. (1982) The preservation of ultrastructure and antigenicity. J. Microsc. **127:** RP5-RP6.

59. Palade, G.E. (1952) A study of fixation for electron microscopy. J. Exptl. Med. **95:** 285-309.

60. Pease, D.C., Baker, R.F. (1948) Sectioning for the electron microscope. Proc. Exptl. Biol. Med. **67:** 470-474.

61. Pease, D.C., Peterson, R.G. (1972) Polymerizable glutaraldehyde-urea mixtures as polar, water-containing embedding media. J. Ultrastruct. Res. **41:** 133-159.

62. Peterson, R.G., Pease, D.C. (1970) Polymerizable glutaraldehyde-urea mixtures as water-soluble embedding media. Proc. 28th Ann. Conf. EMSA, p. 334.

63. Plattner, H., Bachmann L. (1982) Cryofixation: a tool in biological ultrastructural research. Int. Rev. Cytol. **79:** 237-304.

64. Porter, K., Blum, J. (1953) A study in microtomy for electron microscopy. Anat. Rec. **117:** 685-710.

65. Rampley, M.K., Morris, A. (1972) A rapid method for polyester embedding. Proc. 5th. Eur. Conf. Electron Microsc., Manchester, p. 224.

66. Richards, A.G., Anderson, T.F., Hance, R.T.

(1942) A microtome sectioning technique for electron microscopy illustrated with sections of striated muscle. Proc. Soc. Exp. Biol. Med. **51:** 148-152.

67. Ringo, D.L., Brennan, E.F., Cota-Robles, E.H. (1982) Epoxy resins are mutagenic: implications for electron microscopists. J. Ultrastruct. Res. **80:** 280-287.

68. Rosenberg, M., Bartl, P., Lesko, J. (1960) Water-soluble methacrylate as an embedding medium for the Preparation of ultrathin sections. J. Ultrastruct. Res. **4:** 298-303.

69. Ryter, A., Kellenberger, E. (1958a) Etude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. Z. Naturf. **13:** 597.

70. Ryter, A., Kellenberger, E. (1958b) L'inclusion au polyester pour l'ultramicrotomie. J. Ultrastruct. Res. **2:** 200.

71. Sabatini, D.D., Bensch, K., Barrnett, R.J. (1962) New means of fixation for electron microscopy and histochemistry. Anat. Rec. **142:** 274.

72. Sabatini, D.D., Bensch, K., Barrnett, R.J. (1963) Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biol. **17:** 19-58.

73. Sitte, H. (1987) Cryofixation without pretreatment at ambient pressure. In: Cryotechniques in Biological Electron Microscopy (R.A. Steinbrecht and K. Zierold, eds.), pp. 87-113, Springer-Verlag, Berlin.

74. Sjöstrand, F.S. (1951) Freeze-drying of tissues for cell analysis by light and electron microscopy. In: Freezing and Drying (R.J.C. Harris, ed.), pp.177-188, Inst. Biol., London.

75. Spurr, A.R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26:** 31-43.

76. Stäubli, W. (1960) Nouvell matière d'inclusion hydrolysoble pour cytologie éléctronique. C.R. Acad. Sci. **250:** 1137.

77. Van Harreveld, A., Crowell, J. (1964) Electron microscopy after rapid freezing on a metal surface and substitution fixation. Anat. Rec. **149:** 381-385.

78. Voelz, H. and Dworkin, M. (1962) Fine structure of *Myxococcus xanthus* during morphogenesis. J. Bact. **84:** 943.

79. Winborn, W.B. (1964) Epoxy embedments for electron microscopy. Anat. Rec. **148:** 422

80. Winborn, W.B. (1965) Dow epoxy resin with triallyl cyanurate, and similarly modified Araldite and Maraglas mixtures, as embedding media for electron microscopy. Stain Tech. **40:** 227.

81. Wischnitzer, S. (1970) Introduction to Electron Microscopy, 2nd edition, Pergamon press, New York.

82. Wolosewick, J.J. (1980) The application of polyethylene glycol (PEG) to electron microscopy. J. Cell Biol. **86:** 675-681.