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Designing Low, Intermediate or High Viscosity Embedding Media Utilizing Novel Resin/ Anhydride/ Catalyst Combinations

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Epoxy resins are used widely as embedding media because of their proven ability to preserve morphology, minimally extract cell components, infiltrate tissues well, polymerize uniformly without shrinkage, produce favorable sectioning and staining qualities, and withstand the heat and vacuum of the microscope column. Epon 812 satisfied these mandates well and served as the hallmark of epoxy resins for many years[1] . Several epoxies have been introduced in recent years as generic replacements for Epon, since this important epoxy no longer is available. This work examines several of the epon facsimiles in terms of the aforementioned features. The resins were used to prepare low, intermediate, or high viscosity embedding media alternatives by utilizing resins, anhydrides, and catalysts in different combinations so as to produce a final mixture with the particular characteristics necessary to accommodate exact needs.

Individual epoxy resins and amine catalysts (Tables I and II) were tested for **Flow time FT, Volume Flow Rate (VFR)**, and **Viscosity (cp)** by aspirating a known volumen (9ml) of the particular component into a vertically oriented capillary viscosimeter. The material then was allowed to exit freely and the average **FT** (time necessary for 9ml to flow) was recorded. Also, the **VFR** (ml of material flowing per sec) was measured. Because **viscosity** is a correlate of **FT** and **VFR**, **cp** was calculated by using the Hagen-Poiseville relation for laminar flow as it applies to fluids. Embed 812, LX 112, and Polybed 812 were prepared in combination with different anhydrides and catalysts, and their **VFR** and **cp** was determined as a function of time (Tables III, IV, V). A variety of soft tissues were processed according to usual methods and embedded in the various media. Tissues were infiltrated for 30 min in 1: 1 alcohol/resin and 3-6 hrs in full resin. The blocks were hardened overnight @70°C, sectioned, and examined microscopically.

Embed 812 displayed the fastest **average FT , VFR** and thus the lowest **cp** of the replacements (4.1 cp, Table I), while Eponate 12 was the slowest and most viscous compound (20.8 cp, Table I). Amongst the catalysts (Table II), BDMA possessed the fastest **average FT, VFR,** and the lowest **cp** (0.84 cp). DMP-30 flowed much more slowly, thus its **cp** was many times higher (25.0 cp). Embed 812 always yielded the lowest viscosity medium when combined with NSA/NMA and BDMA (22.5 cp @60 min, Table III). Polybed 812, in combination with the traditional (and highly viscous) components DDSA, NMA, and DMP-30 always produced a mixture with the slowest **average FT, VFR,** and **cp** (185.2 cp @60 min; Table V). LX 112 was intermediate in viscous characteristics when mixed with NSA/NMA and the third catalyst, DMAE (Table IV).

Tissues embedded in the various media sectioned well and demonstrated excellent strength in the microscope column. These results, coupled with past work [2,3,4,5] , offer evidence that the Epon substitutes are versatile compounds that are very compatible with alternative anhydrides and catalysts. Thus, microscopists easily can design embedding media exhibiting a wide range of viscosity characteristics, from low to intermediate to high, and that would be suited to special needs.

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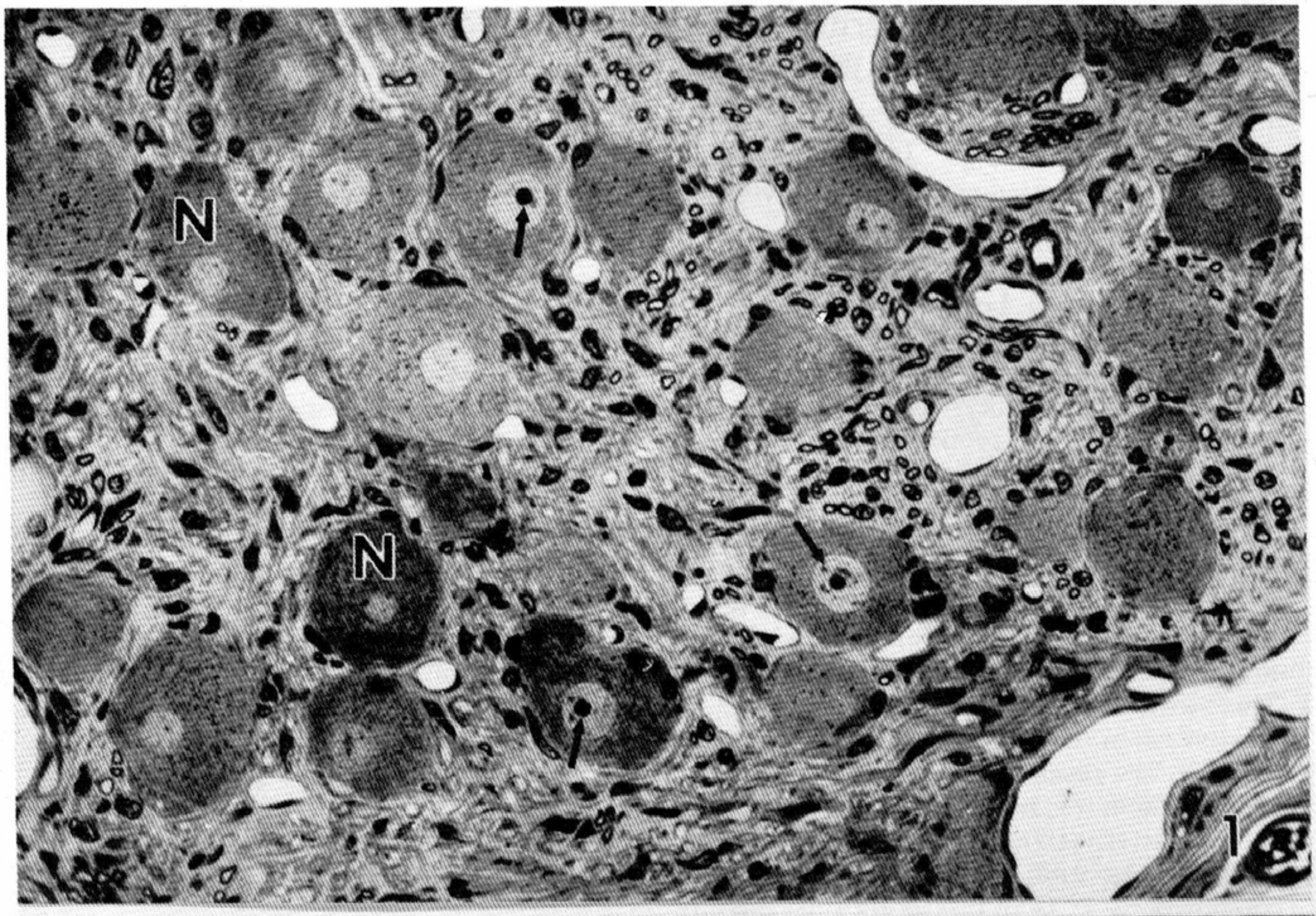
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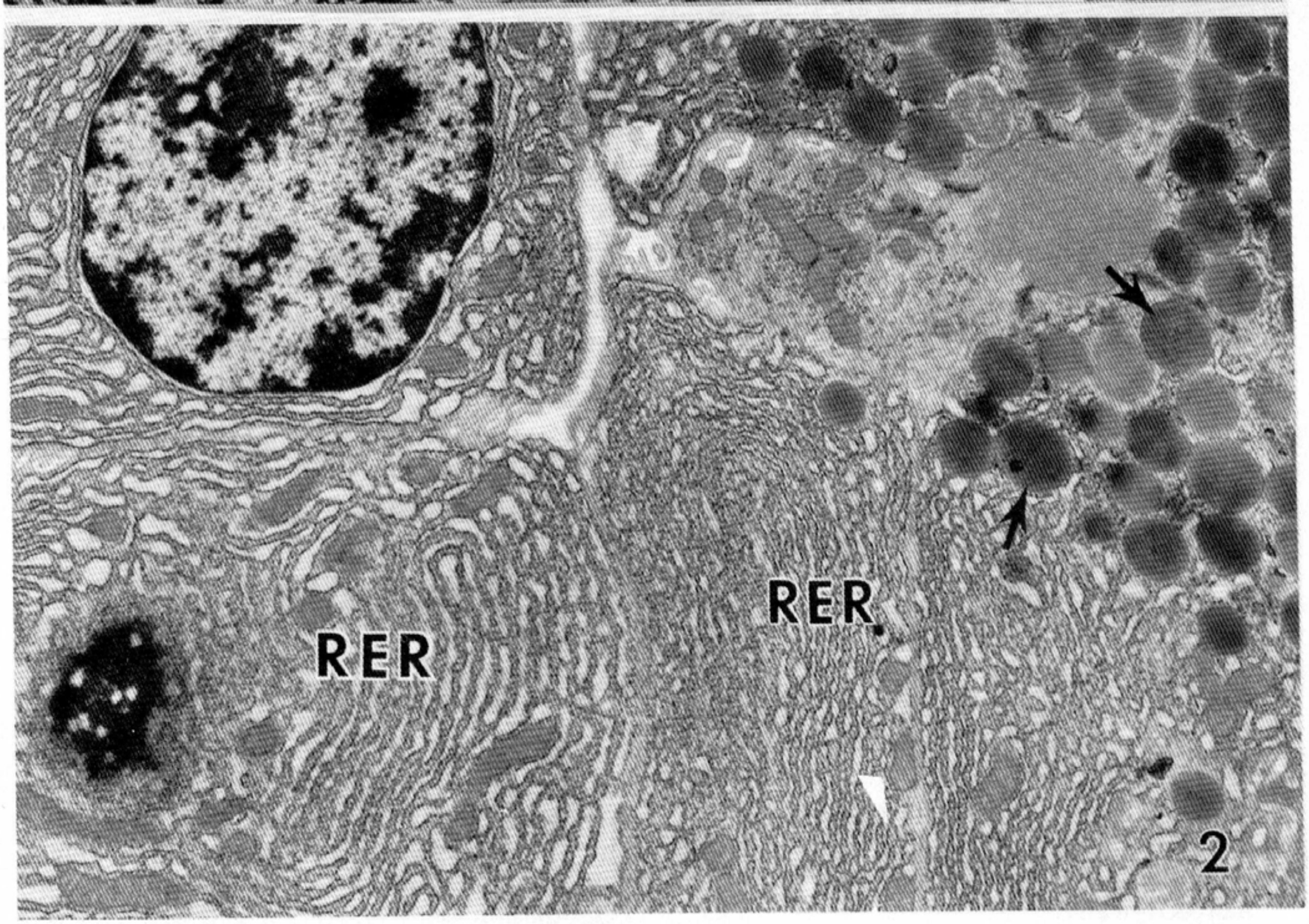
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TABLE I: EPOXY RESIN CHARACTERISTICS - @ 25°C

	Flow Time	Volume Flow Rate	Viscosity (centipoise)
Embed 812	16.81 sec	0.535 ml/sec	4.1 cp
Pelco Medcast	19.96	0.451	5.4
Lx 112	26.83	0.335	7.2
Polybed 812	32.26	0.279	9.1
Eponate 12	**1:10:13 min**	**0.128**	**20.8**

TABLE II: CHARACTERISTICS OF AMINE CATALYSTS - @ 25°C

A (Amine)	BDMA	DMAE	DMP-30
B (Avg FT)	4.57 sec/9ml	5.10 sec	2:11:96 min
C (VFR)	1.97 ml/sec	1.76 ml/sec	0.682 ml/sec
D (cp)	**0.84 cp**	**0.96 cp**	**25.0 cp**

TABLE III: VISCOSITY AND HARDENING CHARACTERISTICS EMBED 812 WITH NSA/NMA/BDMA @ 25°C - (LEAST VISCOUS MIXTURE)

Trial Time	Flow Time	Volume Flow Rate	Viscosity (centipoise)
5 min	51.16 sec	0.1759 ml/sec	10.8 cp
30	1:24:92 min	0.1052	18.0
60	**1:46:45**	**0.0837**	**22.5**

TABLE IV: VISCOSITY AND HARDENING CHARACTERISTICS LX 112 WITH NSA/NMA/DMAE @ 25°C - (INTERMEDIATE VISCOSITY)

Trial Time	Flow Time	Volume Flow Rate	Viscosity (centipoise)
5 min	1:30:08 min	0.0999 ml/sec	28.5 cp
30	3:58:23	0.0378	75.4
60	**6:29:53**	**0.231**	**123.4**

TABLE V: VISCOSITY ANDD HARDENING CHARACTERISTICS POLYBED 812 WITH DDSA/NMA/DMP-30 @ 25°C - (MOST VISCOUS MIXTURE)

Trial Time	Flow Time	Volume Flow Rate	Viscosity (centipoise)
5 min	2:43:35 min	0.0551 ml/sec	45.0 cp
30	7:21:59	0.0204	121.7
60	**11:10:98**	**0.0134**	185.2

Fig. 1: A light micrograph illustrating neurons (N) with prominent nuclei and nucleoli (arrows) from the inferior mesenteric ganglion of a young New Zealand white rabbit. Material was embedded in Embed 812/NSA/NMA/BDMA low viscosity medium. One micron section stained with toluidine blue. Magnification: X 1,800 approximate.

Fig. 2: Electron micrograph showing exocrine pancreas from a young New Zealand white rabbit. Cells are rich in rough-surfaced endoplasmic reticulum (RER) and zymogen granules (arrows). Material embedded in LX 112/NSA/NMA/DMAE intermediate viscosity medium. 800 Å section double-stained with lead citrate/uranyl acetate.
Magnification: X 18,000 approximate