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Stempak J G & Ward R T. An improved staining method for electron microscopy. *J. Cell Biol.* **22**:697-701, 1964.
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Uranyl acetate dissolved in methanol provides a faster, cleaner, and more intense stain for transmission electron microscopy than does aqueous solutions It is effective for use in high-voltage electron microscopy and as a mordant in double-stain procedures, and it may stain relatively unreactive tissues more effectively. [The *SCI*® indicates that this paper has been cited in over 705 publications since 1964]

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"When I arrived at the State University of New York in 1962, I began a careful scrutiny of tissue preparation for electron microscopy. During that period, I dehydrated tissues with graded methanol solutions because I had duplicated some experiments of Bahr and others that indicated that less tissue shrinkage took place during methanol dehydration than during ethanol dehydration.[1] It occurred to me that *en bloc* staining by a solution of uranyl acetate in methanol might eliminate the need to stain sections, so various solutions of uranyl acetate in 100 percent methanol were made, and the experiments attempted.

"The first experiments in *en bloc* staining of adult rat liver with solutions of uranyl acetate in methanol yielded sections in which all elements were heavily stained, and the resulting micrography was uniformly dark. Micrographs of sections from unstained tissues that were routinely prepared and stained with solutions used for *en bloc* staining yielded well-stained material in very short time periods, and the sections were uncommonly clean. It seemed sensible to document what was obviously successful, so we stained other tissues and embedments with similar success and reported on the stain. Refinement of the *en bloc* staining experiments was put off until 'later,' a time much like 'tomorrow' since it seems not to come.

"The only modification in technique we have made over time is to insert the lid of a histology tissue-preparation capsule (we use Tissue-Tek') into the Stender dish of solution and allow it to submerge. We place the grids in a vertical position in the perforations, which makes retrieval much easier.

"The technique enjoyed modest success for conventional transmission electron microscopy, and in 1973, Carasso, Delaunay, Favard, and Lechaire judged it a superior method for high-voltage electron microscopy.[2] Thick sections, re-embedded and sectioned perpendicular to their faces by the aforementioned authors, have confirmed our hypothesis that the excellent staining properties of the methanolic stain were due to better penetration of the epoxy sections.

"Today, most investigators believe that adequate contrast can be achieved only by double staining: uranyl acetate followed by lead. Our method provides sufficient contrast if used alone,[3,4] provided that time is varied to suit the particular embedding material. Sections from some Epon and Spurr blocks require up to 30 minutes, while the more resistant Maraglas may require 90 minutes. We prefer to avoid lead staining altogether so that we do not have to be concerned about the beam-induced granular coalescence and growth of lead particles during the observation of what turns out to have been one's best sections.

"However, for those who require double stain ing, we suggest methanolic uranyl acetate fol lowed by Paley's lead stain. For ordinary use, lead staining time can be reduced to seconds, thus min imizing exposure to atmospheric CO_2, the princi pal cause of contamination. With longer exposure to lead, very intense staining of membranes oc curs, rendering better viewing at low magnifica tion, but excessive granularity at high magnifica tion. Further, tissues or cells that are inherently difficult to stain may be adequately stained by this sequence.

"The publication has been well cited because the method stains rapidly and well, and is cleaner. The stain penetrates thick sections more deeply, making it excellent for high-voltage electron microscopy, and achieves a less granular image in high-resolution micrographs.[5] For those who require double staining, its use as the mordant yields superior results. For recent work in this field, see reference 6."

1. **Bahr G F, Bloom G & Friberg U**. Volume changes of tissues in physiological fluids during fixation in osmium tetroxide or formaldehyde and during subsequent treatment. *Exp. Cell Res.* **12**:342-55. 1957. (Cited 90 times since 1957.)
2. **Carasso N**, **Delaunay M C, Favard P & Lechaire J P**. Obtention et coloration de coupes épaisses pour la microscopie électronique a haute tension. *J. Microscopie* **16**:257-68. 1973.
3. **Ward R T.** The origin of protein and fatty yolk in *Rana pipiens.* III. Intramitochondrial and primary vesicular yolk formation in frog oocytes. *Tissue Cell* **10**:515-24. 1978.
4. ..... The origin of protein and fatty yolk in *Rana pipiens.* IV. Secondary vesicular yolk formation in frog oocytes. *Tissue Cell* **10**:525-34, 1978.
5. **Stempak J G & Laurencin M**. High resolution microscopy of intracellular membranes. *J Microscopie* **9**:465-76. 1970.
6. **Ward R T.** The origin of protein and fatty yolk in *Rana pipiens.* V. Unusual paracrystalline configurations within the yolk precursor complex. *J. Morphology* **165**:255-60, 1980.