Formaldehyde as a Fixative for Light and Electron Microscopy

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Since Blum discovered its hardening properties in 1893, formaldehyde has become the most widely used fixative in the world for specimens to be examined by light microscopy. However, since most commercial preparations of formaldehyde contain methanol, a protein precipitant, formaldehyde has been considered an unsatisfactory fixative for tissues to be examined by electron microscopy. In 1973, Carson *et al.* described a parallel study comparing the electron microscopic results of fixation with paraformaldehyde vs. formaldehyde.[1] They found that there was no difference in the preservation of ultrastructural morphology provided that the buffer systems were identical. In 1976, McDowell and Trump described a fixative combining commercial formaldehyde and glutaraldehyde (4CF-1G).[2] Both of these fixatives are dual purpose fixatives and preclude the selection of tissue for electron microscopy prior to fixation. They can both be prepared in large quantities and used for routine surgical specimens. The fixative containing formaldehyde alone does not need to be refrigerated and is stable for months; whereas, the formaldehyde-glutaraldehyde mixture should be refrigerated. The 4CF-1G solution will show a 0.2 to 0.3 unit drop in pH and will turn cloudy in 4 to 8 weeks. Although tissue does not need to be preselected for electron microscopy, very thin sections should be taken from the periphery of fixed thicker tissues for ultrastructural studies. Trump and Jones reported no change in ultrastructural preservation after storage for 36 months in either of these fixatives.[3]

In our investigations, we initially looked at the differences in the ultrastructural preservation of blood and bone marrow fixed in the usual phosphate buffered formaldehyde found in most histopathology laboratories, in a modified Millonig phosphate buffered formaldehyde, in a neutralized (with marble chips) formaldehyde, and in an acetate buffered formaldehyde. Modified Millonig solution is prepared with sodium monobasic phosphate and sodium hydroxide, which when combined in solution will immediately give an equilibrium between sodium monobasic and sodium dibasic phosphates. The amount of alkali can be varied so that the pH can be adjusted between 5.4 and 8.0 without changing the tonicity of the fixative.[4] The modified Millonig solution gave superior results and so this solution was chosen for the parallel studies.[5] The usual phosphate buffered formaldehyde solution has a milliosmolality of approximately 161 exclusive of the formaldehyde and a pH of 6.85; whereas, the Millonig preparation has a milliosmolality of 290 and a pH of 7.2-7.4. The latter is very close to the milliosmolality of plasma and probably accounts for the superior ultrastructural preservation seen. That there is less cytolysis with the Millonig preparation is also apparent on light microscopic preparations. Because of the increased phosphate concentration, the concentration of the first alcoholic solution used for processing should not exceed 65% or the phosphate salts will precipitate.

In an earlier study, we had investigated the effect of varying both the buffer system and the concentration of paraformaldehyde.[6] Blood and bone marrow were selected for this study because the fixative is immediately in intimate contact with the cells and therefore differences in penetration rates, block sizes, and intrinsic tissue variances were eliminated. Formaldehyde has not been considered to be osmotically active by some investigators, and we found that varying the paraformaldehyde concentration between 0.5% and 4% exerted very little effect on the ultrastructure of blood or bone marrow cells.[7,8] However a very noticeable effect on ultrastructure was noted when the buffer system was var-

ied. We studied s-collidine, cacodylate, and phosphate buffer systems, and found that the Millonig phosphate buffer gave vastly superior results over the other two systems. As a result of this study, in 1972 we changed from an s-collidine buffer system to a phosphate buffered paraformaldehyde for all routine electron microscopy studies, and in 1973 we changed to a phosphate buffered formaldehyde solution. Since that time, the modified Millonig formaldehyde solution has be used to fix all specimens for electron microscopy, including kidney biopies, and the results have been excellent. ■

1. Carson FL, Martin JH, and Lynn JA: Formalin fixation for electron microscopy. *Am J Clin Path* 59:365-373. 1973.
2. McDowell EM and Trump BF: Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Path Lab Med* 100:405-414, 1976.
3. Trump BF and Jones RT: *Diagnostic Electron Microscopy*. New York, John Wiley & Sons. pgs.115-130. 1978.
4. Pease DC: *Histological Techniques for Electron Microscopy*, 2nd ed. New York. Academic Press. pgs. 39-40. 1964.
5. Carson FL: *Histotechnology: A Self-Instructional Text*, 2nd ed. Chicago, ASCP Press. pgs 11-12. 1997.
6. Carson FL, Lynn JA, and Martin JH: Ultrastructural effect of various buffers, osmolality, and temperature on paraformaldehyde fixation of the formed elements of blood and bone marrow. *Texas Rep Biol Med* 30:125-142, 1972.
7. Bernard GR and Wynn GG: Weight responses of tissue slices and albumin-gelatin gels during formaldehyde fixation with observation on the effect of pH. *Anat Rec* 150:463-472. 1964.
8. Maunsbach AB: The influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. Effects of varying osmolality, ionic strength, buffer system, and fixative concentration of glutaraldehyde solutions. *J Ultrastruct Res* 15:283-309. 1966.