

# This Week's Citation Classic™

**Karnovsky M J.** A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27:137-8A, 1965.

[Department of Pathology, Harvard Medical School, Boston, MA]

A formaldehyde-glutaraldehyde fixative was formulated that produced useful fixation of a wide variety of cells and tissues. It was surmised that the formaldehyde would penetrate faster than the glutaraldehyde and temporarily stabilize structures that are subsequently more permanently stabilized by the slower-penetrating, but more efficiently cross-linking, glutaraldehyde. The high osmolality of the fixative, due to the aldehydes, did not usually appear to be a matter of much moment, insofar as cell shrinkage was concerned. [The *SCI*® indicates that this abstract has been explicitly cited in over 4,505 publications. It is probably the most-cited abstract in the history of science.]

Morris J. Karnovsky  
Department of Pathology  
Harvard Medical School  
Boston, MA 02115

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In the 1960s, electron microscopists were greatly concerned with improving their techniques, including those for fixation. Many of these efforts proceeded empirically, the endpoint being a matter of judgment as to the "life-like" preservation of cells and tissues. There were also those who systematically paid great attention to the osmolality, pH, ionic composition, and so on, of the fixative. It seemed to me that, as the tissues interacted with the fixative, the osmotic properties of cells would change considerably. The fixative would be diluted by tissue fluids, and that aldehydes, being lipid soluble, should not be counted as contributing markedly to the overall osmolality as far as effects on cell volume were concerned. The vehicle (i.e., the buffer) and added constituents (such as salts and macromolecules) would be more important. I further surmised that formaldehyde would penetrate faster into blocks of tissue than the

more effective but larger cross-linker, glutaraldehyde.

I thus concocted a buffered formaldehyde-glutaraldehyde fixative, which, upon use, proved to be highly efficacious in fixing a wide variety of tissues. The original formulation was hypertonic (2,010 milliosmolar!), and, although this worked well on tissue blocks, it was recommended that diluted solutions were more suitable for fixing monolayers and cell suspensions and for use in organ perfusion. Nevertheless, the diluted solutions are also hypertonic, and as subsequent work by others (reviewed in references 1 and 2) produced evidence that the aldehydes are not themselves osmotically active, my original provocative and tongue-in-cheek formulation of a fixative of high osmolality now seems to have been fairly justified.

As I did not consider the presentation of this fixative to warrant a full-scale paper, it has only been published in abstract form—in fact, it was merely "read by title" and not formally presented at the Fifth Annual Meeting of the American Society for Cell Biology in 1965.

I am somewhat surprised that, despite this rather pauperitic mode of presentation, the fixative has had widespread use and is colloquially well known as "Karnovsky's fix," or "Karnovsky's half-and-half," the latter referring to the most common dilution in which the fixative is currently used. No doubt, most biologists feel anxiety at the idea of plunging tissues into the original hyperosmotic formulation!

I consider the scientific content of the original paper trivial, but like many technical tricks, the fixative has obviously proved useful to many, even though a factual underpinning for the rationale offered for its development was, and is, largely lacking. I empathize with Sir Arthur Sullivan of Gilbert and Sullivan fame: he too, would have rather been recognized and remembered for his "serious" music than for the popular operettas.

1. **Glauert A M.** Fixatives. (Glauert A M, ed.) *Practical methods in electron microscopy*. New York: American Elsevier, 1974. Vol. 3. p. 5-65.
2. **Hayat M A.** Fixation. (Hayat M A, ed.) *Principles and techniques of electron microscopy: biological applications*. New York: Van Nostrand Reinhold, 1970. Vol. 1. p. 13-105.