

# Citation Classics

Venable J H & Coggeshall R. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biology* 25:407-08, 1965.

**One of the more stable and reliable stains, commonly called the lead citrate stain or Reynolds' stain, is made by mixing lead nitrate and sodium citrate in distilled water, allowing time for lead citrate to form, then adding sodium hydroxide to raise the pH of the solution to 12. This paper reports the use of a commercially available lead citrate to eliminate the preparatory steps of Reynolds' procedure, thus saving considerable time. [The SCI® indicates that this paper was cited 2,120 times in the period 1961-1975.]**

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"A bit of sloth and a desire for speed prompted development of the simplified lead citrate stain. Certainly there was no meditation over the possibilities of creating anything original or of wide usefulness. A postdoctoral fellowship in the early 1960's to learn electron microscopy placed me across the hall from Ed Reynolds in Don Fawcett's department at Harvard. Reynolds had just concocted his new lead citrate stain, which solved much of our problems with lead precipitates. My new aggravation, however, was the elapsed one hour or more from the decision to make a new batch of Reynolds' formulation to the examination of the stained sections. Although the new stain was relatively stable, I seemed forced to make a new batch, hoping to eliminate any staining problems, each time I looked at my most recent floundermgs at microtomy

"A possible escape from this frustration stemmed from a thought that the chemical industry should be able to supply lead citrate more economically than we microscopists could ever achieve by tinkering individually

The ingredients of Reynolds' formulation other than lead citrate, namely high pH and secondary reaction products, could be supplied directly and quickly. The secondary reaction products, however, could interfere with the staining, and thus be superfluous. To my surprise lead citrate was listed in K & K Chemical's catalogue

"I talked *Sus Ito* out of the money to order the minimum quantity, 50 grams, a staggering amount for the use in mind. By the time it arrived, I had forgotten about it, Reynolds' stain serving me nicely. I stijn gave the simplified mixture a first trial. It yielded mainly despair. Twenty minutes in a high pH solution of lead citrate--the then average staining time--left the sections globbed with coarse precipitates. But the structure was stained. Eventually the staining time was reduced to seconds, the lead citrate concentration cut considerably and--eureka--we had a stain that could be made in ten minutes. In three more minutes the stained sections could be in the microscope

"The use of this simple lead citrate modification did not sweep the local laboratories by any measure. Richard Coggeshall, my co-author, working on the nervous system of the leech, decided early to try it and gave it its first real test on a variety of tissues and spread its use in Sanford Palay's laboratory. By the time I finished my postdoctoral so many investigators there were using it that Coggeshall encouraged publication. My response was that no self-respecting journal would publish such an empirical, mundane recipe in the face of Reynolds' well conceived paper. Coggeshall's rebuttal was that we all were mailing the formulation to friends; placing it in the literature was a more efficient approach. *Stain Technology* and the *journal of Histochemistry and Cytochemistry* rejected it dutifully on perfectly logical grounds.

"After some months Sanford Palay's influence brought the formulation to print in the *journal of Cell Biology* and a 'Citation Classic' resulted, an extravagant honor for such minor intellectual effort."