CC/NUMBER 32
AUGUST 10,1981

This Week's Citation Classic

Reynolds E S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208-12, 1963.
[Dept. Anatomy, Harvard Medical School, Boston, MA]

A method for staining ultrathin sections for electron microscopy with solutions of lead citrate at high pH is described. Lead citrate solutions are stable for long periods of time and do not significantly contaminate the sections with unwanted precipitate. The physical-chemical basis of this staining is discussed. [The *SCI*® indicates that this paper has been cited over 13,395 times since 1963.]

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June 26, 1981

"This oft cited 'Brief Note' in the *journal of Cell Biology* describes a method for the staining of ultrathin sections for electron microscopy. It was really a rather simple thing to do —stain sections with lead ions where the lead is maintained in a complex with a multivalent organic anion (citrate) at a pH where the hydrated lead ion is positively charged and possibly exists in a polymeric complex. Complex formation between the lead cation and citrate multi-anion prevents its precipitation as lead carbonate and stains tissue sites (presumably phosphate groups of structural lipids RNA and DNA) which have a greater affinity for lead ion than citrate. Sound good? Yes, but unfortunately—or fortunately— the hypothesis stated above was devised after the fact to explain the phenomenon. Much of the hypothesis remains unproven, but the stain works!

"At the time that I stumbled across this phenomenon in 1961, I was a research fellow in the department of anatomy at Harvard Medical School Electron microscopy was an all consuming technical four *de force* One had to be a longtime apprentice The chairman of the department cut his sections at home at night! Long hours were spent in the lab discussing the best ways of making glass knives. Milk bottle shards, heat broken knives, plier broken knives all had their advocates. Days and weeks were spent cutting the 'perfect' section. Staining with lead reminded one of the procedures of the medieval alchemist. Super pure lead oxide was boiled in 'CO_2 free' aqueous NaOH in a CO_2 free atmosphere. The product was diluted with CO_2 free water and sections stained for exact times under the most scrupulously clean conditions in an atmosphere elaborately purged of CO_2 Fresh stain had to be made daily and then was good only for several hours —if it worked at all. Yet, in spite of the most stringent precautions many 'perfect' sections were ruined by coarse black precipitates —almost always diagnosed as 'lead carbonate.' Having come from a postdoctoral fellowship in biophysics where I was aware of metal organic ligand binding constants, one morning I said something like, 'Why don't we stabilize the lead in solution with an organic ligand which will release it to binding sites in tissues, but which will keep it from reacting with CO_2 in the atmosphere.' Wiser heads nodded skeptically but said, 'Go ahead, but I don't think it will work.' The first vial of stain was ready within an hour. The effect was amazing. After a few days Fawcett came to me and said that I really must pubiish the method —and fast. So I did. The first vial of stain lasted three years. Sheer serendipity!

"By now the article has been cited several thousand times. Indeed, it has become such a classic that many authors no longer cite it at all. But one of my 'pet peeves' is those authors who, if they must cite it, cite it incorrectly. These people have become legion. In 1979 —the last year for which there is an annual *Science Citation Index*® in our library —the article was miscited 22 different ways and the most frequent form of miscitation was repeated 14 times. Thus, I am often miscited more for this article than I am correctly cited for some of the articles which I consider my more significant publications.* Sic *transit gloria*!"

"Variant forms of citations to E. S Reynolds's article will be corrected in the new *Science Citation Index*® 1975-1979 cumulation.