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Karnovsky M J. Simple methods for "staining with lead" at high pH in electron microscopy. *J. Biophys. Biochem. Cytol.* 11:729-832, 1961.
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This paper presented a highly alkaline lead-containing solution for "staining" thin sections in order to enhance contrast in the electron beam. It was proposed that the high pH stabilized the solution and prevented the formation of contaminating precipitates on exposure to air. [The *SCI*® indicates that this paper has been cited in more than 1,620 publications.]

Lead Staining Without Dross

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In order to obtain sufficient contrast in thin sections of biological material, electron microscopists have long needed to "stain" with solutions of heavy metals, increasing the electron opacity of structures in the specimen. M.L. Watson[1] found that an alkaline (pH 8.5) solution of lead hydroxide stained well, and his method was widely used. However, there was an unfortunate and frustrating propensity for contamination of the section on exposure to air during staining, due to the formation of precipitates, presumably of lead carbonate. Despite modifications of the original formula and the invention of ingenious devices for preventing exposure to air, contamination was not totally eliminated.

Working late one night in the laboratory, I was highly irritated by the contamination I was getting on my sections, so I started to see if I could stabilize the solution by various maneuvers. As a test system, I put drops of modified stain on dental wax and scored the time elapsed for a milky precipitate to occur on exposure to air. Soon, the laboratory benches, desks, windowsills, etc., were occupied, much to the vexation of my colleagues when they arrived in the morning!

I found that highly alkaline (pH 11.5) solutions of lead salts yielded relatively stable solutions that stained cleanly. I thought that lead would be present in the highly alkaline solutions as an hydroxide complex anion [plumbite ion, Pb $(OH)_3$. or Pb $(OH)_{4,,}$].[2] Lead monoxide, like lead hydroxide, is amphoteric and acts as an acid anhydride, forming the plumbite ion on treatment with a base. I therefore found in the chemical stores an old bottle of lead monoxide and dissolved it in NaOH. The resultant stain worked very well. It was certainly easier and less messy to use dry lead monoxide powder than to make lead hydroxide, as in the Watson formula.

My stain was widely used. However, it was not completely foolproof and was gradually superseded by alkaline solutions of lead citrate, in which it is believed that the stain is stabilized by chelation of the lead.[3]

With increasing pH levels, anionic groups in the section became more completely ionized, thus binding Pb^{2+}. I suggested that this was not the basis for staining at the high pH (11.5) of my solutions, where it is unlikely that positively charged tissue groups are available for binding the negatively charged plumbite ion. I suggested another mechanism of staining—namely, hydrogen bonding of the plumbite to tissue components—and adduced some evidence in support of this hypothesis. However, $[Pb(OH_2)Pb]^{2+}$ also exists in alkaline solution.[2] Various components of tissues probably bind lead by different mechanisms, as discussed in references 4 and 5.

1. **Watson M L.** Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium. *J. Biophys. Biochem. Cytol.* 4:727-9. 1958. (Cited 665 times.)
2. **Remy H.** *Treatise on inorganic chemistry.* New York: Elsevier, 1956. Vol. 1.
3. **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. (Cited 18,765 times.) [See also: **Reynolds E S.** Citation Classic. *Current Contents/Life Sciences* 24(32):16. 10 August 1981. Reprinted in: *Contemporary classics in the life sciences. Volume 1: cell biology.* (Barrett J T, ed.) Philadelphia: ISI Press. 1986. p. 6.]
4. **Hayat M A.** *Principles and techniques of electron microscope. Biological applications.* Boca Raton. FL: CRC Press. 1989.
5. **Lewis P R & Knight D P.** Staining methods for sectioned material. (Glauert A M. ed.) *Practical methods in electron microscopy.* New York: Elsevier. 1988. Vol. 5. Part I.

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