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A STABLE LEAD STAIN BY MODIFICATION OF SATO'S METHOD

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Since the first report by Watson[2], various lead stains have been developed in order to increase contrast and reduce contamination in sections for electron microscopy. One of the disadvantages of lead stains so far developed is that the staining solution is apt to produce a precipitate of lead carbonate, resulting in difficulty in long-term storage of lead stains. We will recommend a new stable lead solution which is free from precipitate over one year at room temperature. A stock lead solution was made up as follows;

Anhydrous lead citrate	$Pb(C_8H_5O_7)_2$	0.20 g
Lead nitrate	$Pb(NO_3)$	0.15 g
Lead acetate	$Pb(CH_3COO)_2 \cdot 3HO_2$	0.15 g
Sodium citrate	$Na_3(C_6H_5O_7) \cdot 2H_2O$	1.00 g
Distilled water		41.0 ml

The above reagents placed in a 50 ml volumetric flask were mixed well to make a yellowish milky solution. Then, the solution was added 9.0 ml of 1 N NAOH and mixed well until the solution became transparent with light yellowish color.

In order to assess the stability, the solution thus prepared was divided into two aliquot parts and each was kept in brown glass bottles with a plastic screw cap. One bottle was left at room temperature, and the other was kept in the refrigerator until use. The solutions from both bottles were checked every 30 days for the presence of precipitate and stainability. Staining of thin sections with the lead solution was carried out by the procedures recommended by Sato. Normal mouse liver, fixed doubly with glutaraldehyde/OsO_4 and embedded in Epon, was used for the experiments.

No precipitates were found either in the solution kept at room temperature or in the refrigerator over one year.

As shown in Fig. 1, a mouse liver cell stained with the lead solution immediately after preparation shows suitable contrast similar to that (Fig.2) stained with the Sato's original solution[1]. Sections from the same specimen were similarly stained with lead solution kept at room temperature for more than 6 months (Fig.3). No contamination was noticed in the sections, even when the solution was used without filtration. While a lot of contamination was observed in the sections when Sato's original solution was used without filtration.

1. Sato, T.: J. Electron Microsc. 17:158, 1968.
2. Watson, H.L.: J. Biophys. Biochem. Cytol. 4:727,1958.