A MODIFIED PROCEDURE FOR LEAD STAINING OF THIN SECTIONS

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Metal impregnation, or "staining," of tissue sections for electron microscopy has become the accepted practice in recent years. Salts of metals of high atomic weight such as uranium, chromium, thorium, lead, or tungsten, among many tested, have been found suitable (6, 1). "Lead hydroxide," as preparted by Watson (6), is now widely used, but this solution is extremely unstable in air and becomes covered by a metallic-appearing film which imparts to the "stained" section a well known deposit of electron-opaque particles and crystals, reducing considerably the percentage of clean areas suitable for micrography (Fig. 1).

Several procedures and devices have been described to lessen these contaminations. Peachey (5), using lead hydroxide, or subacetate, found that if the solution is kept in a syringe with the protecting cap filled with sodium hydroxide to absorb the CO_2 of the atmosphere, contamination is reduced. More recently, a fairly unwieldy apparatus has been suggested for the same purpose (4). Tests performed in our laboratory in which "staining" was attempted in a chamber under continuous nitrogen flow and in the presence of a barium chloride trap were not completely successful, which is to say that contamination continued to be a problem.

A number of other variations have been recommended. Lever (2), *e.g.*, described a method for preparing a "staining" solution by adding potassium hydroxide to a lead hydroxide solution. To dissolve some of the occasionally formed crystals of contamination, the sections are rinsed afterwards for a few seconds in a weak potassium hydroxide solution; this step is very critical because the "staining" is also weakened by the alkali and uniform staining is difficult to achieve.

These various shortcomings of currently available methods have stimulated us to search for a lead salt that would not be affected by the components of the atmosphere.

Among several heavy metal salts tested, the above-mentioned "lead hydroxide" of Watson seemed to be the most effective in "staining." Experiments with the commercially available product showed that this salt would give no impregnation when dissolved in water, or in acids. In alkaline solutions, however, of, *e.g.*, sodium or potassium hydroxide, it "stained" very effectively. Therefore, one can assume that a chemical reaction takes place upon adding the sodium or potassium hydroxide to the lead hydroxide, and one or more newly formed salts are present in the mixture. In fact, in such a solution different products, such as lead oxides and hydroxides, sodium plumbite and hydroplumbite, have been indicated, but their isolation seems to be extremely difficult (3). More important for our purposes was not so much the isolation of the "staining" salt, as to have a solution that can be used without special precautions.

The reasoning which guided us to further experiments was as follows: It is known that when sodium hydroxide is added to a copper sulfate solution, a copper hydroxide is produced. To solubilize this hydroxide and stabilize it as a complex salt, sodium potassium tartrate is added. This reagent is widely used in the clinical laboratory for glucose tests and is named after Fehling. It seemed justified, on the basis of the above reaction, to try adding tartrate to an alkaline lead solution to achieve a similar stabilization of the lead salt.

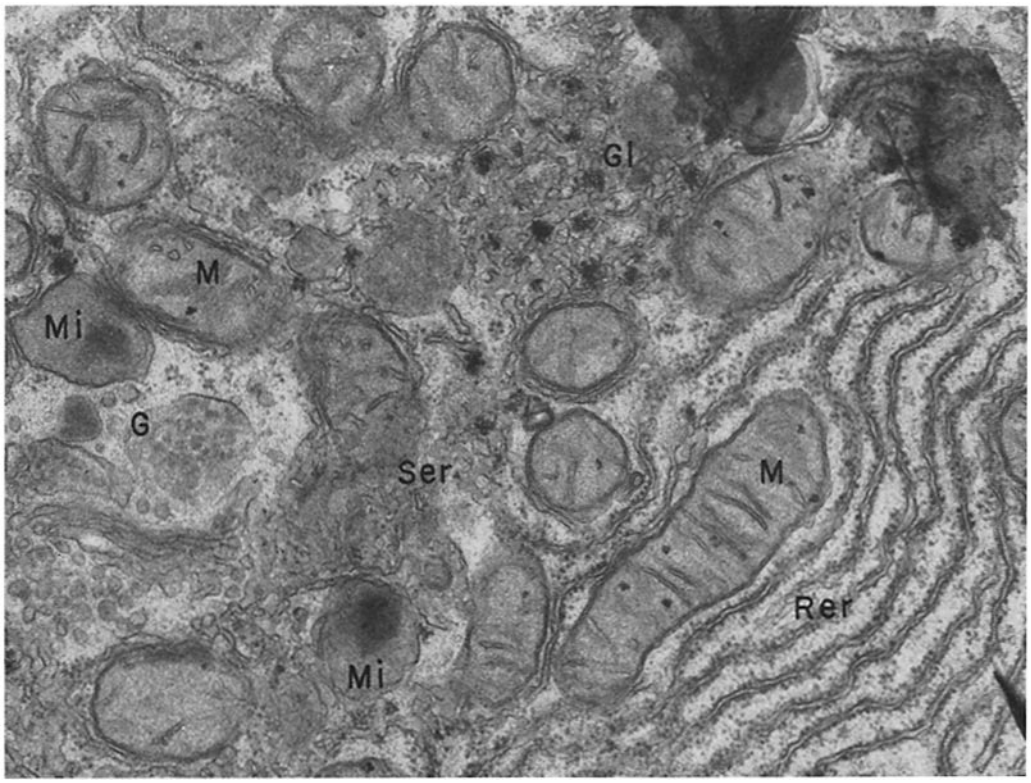
Our experiments showed that when tartrate is added to an alkaline lead hydroxide solution the staining capacity is not greatly reduced and the solution stays clear even when not protected from the air. The next step sought to determine the minimal amounts of sodium hydroxide and tartrate that could be added to the dissolved lead hydroxide to prevent precipitate formation and still achieve good staining. Out of these experiments there evolved the following stock solution containing both, the tartrate and the sodium hydroxide, which upon use is diluted to serve as the solvent for the commercial lead hydroxide. It is stable indefinitely.

NaOH	12.5 gm
K-Na-tartrate	5.0 gm
H_2O to	50.0 ml

In making up the staining solution, 0.5 ml of this stock solution is diluted to 100 ml with distilled

water, heated, and 1 gm commercial lead hydroxide (Amend Drugs, New York City) is added.[1] After cooling, this solution is filtered and should remain clear. It has a pH of about 12.3.

If lead hydroxide is not available, it may be prepared from lead acetate and sodium hydroxide. In making up the "stain" 1 ml of this solution is added to 5 ml of a 20 per cent lead acetate, $Pb(CH_3COO)_2 \cdot 3\ H_2O$, solution, stirred, diluted 5 to 10 times with distilled water, and filtered. This resulting solution is also colorless and clear, and can be assumed to contain the same staining



Key to Abbreviations

G—Golgi components
Gl—glycogen
L—lysosome
M—mitochondrion
Mi—microbody
Rer—rough endoplasmic reticulum
Ser—smooth endoplasmic reticulum

FIGURE 1

Section of rat liver "stained" with a lead hydroxide solution prepared by the method of Watson and showing characteristic evidence of contamination. × 24,000.

For this reaction a similar stock solution has been used, differing only in containing a higher amount of sodium hydroxide:

NaOH	20.0 gm
K-Na-tartrate	1.0 gm
H_2O to	50.0 ml

[1] The lead hydroxide should be protected from the CO_2 of the atmosphere.

salt as that prepared with commercial lead hydroxide.

The two "staining" liquids have proved: (*a*) to "stain" thin sections within a short time (5 to 20 minutes); (*b*) not to damage sections in methacrylate, Epon, or Vestopal; (*c*) to be stable under conditions of room temperature and atmosphere for several weeks; and (*d*) not to contaminate the sections.

The "stain" can be stored in a glass bottle with a dropper, ready for use. The time of impregnation varies: for methacrylate it is from 5 to 10 minutes, and for Epon from 5 to 20 minutes. Tissue embedded in Epon one year earlier and sections cut six months previously were "stained" successfully. The grid with sections is either floated on or immersed in this solution and the following devices are used for this purpose: either a small vial filled with the "stain," or two drops on a paraffin-coated Petri dish. During this time the vial is protected from dust and evaporation by a cover. Afterwards the grid is washed briefly with distilled water by agitation in a beaker or, better, under a jet from a plastic wash bottle, taking care that all of the solution has been washed off from the grid and from the forceps.[2]

[2] The toxicity of lead solutions should be kept in mind and due precautions taken all times.

In comparative studies undertaken with this and the other lead "stains," all conditions were kept as constant as possible; *e.g.*, the thickness of the sections, staining time, film emulsion, and development. Negatives of average density, prepared with Kodak Medium plates, were printed on Kodabromide Paper, grades 2 or 3. The two photographs used in the publication were taken with a Siemens Elmiskop I at 60 kv and the enlargements printed on grade 2 paper. The contrast achieved is considered to be adequate, taking into account the fact that the tissue has been embedded in Epon.

The quality of "staining" achieved with the "lead-tartrate," or the degree of impregnation, seems not very different from these achieved with the original Watson formula, and such slight differences as can be noted between Figs. 1 and 2 have not been observed with sufficient consistency

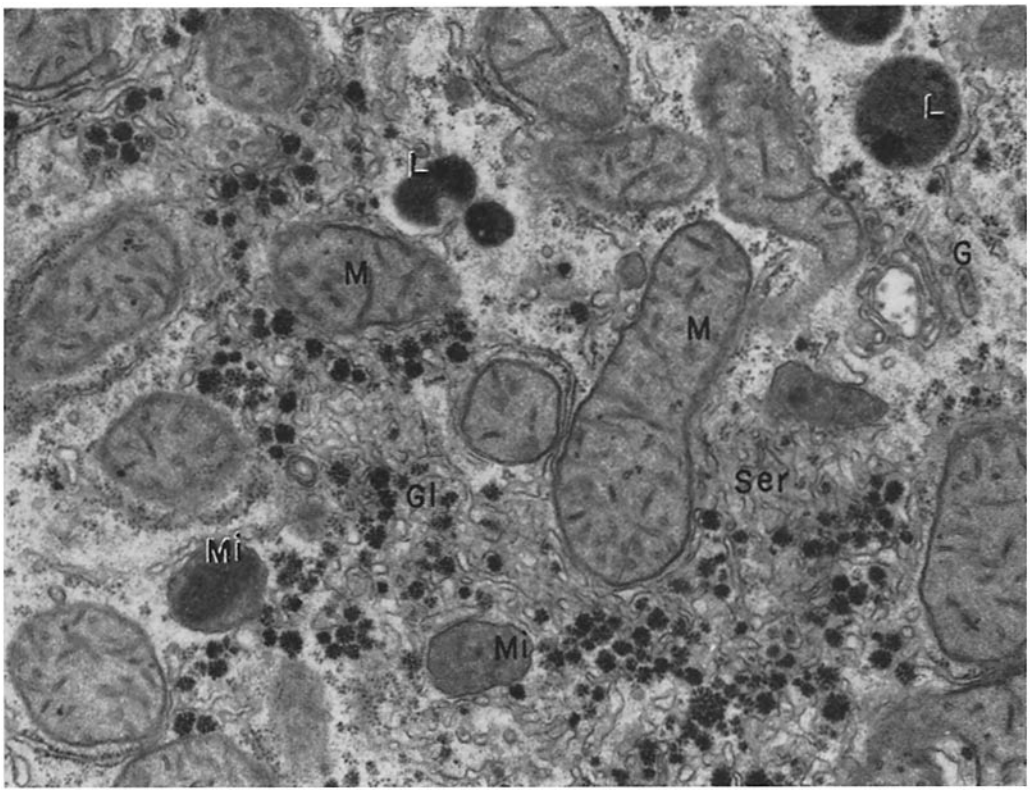


FIGURE 2

The same tissue "stained" with a lead solution containing sodium potassium tartrate. The staining properties are similar and the section is devoid of any contamination. × 24,000.

to convince one that they are not the result of inevitable variations in procedure. At very least, the newly described "stain" provides a range of density and contrast in the preparation which permits the clear identification of all known cell organelles and avoids the nuisance of contamination.

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