A SIMPLIFIED LEAD CITRATE STAIN FOR USE IN ELECTRON MICROSCOPY

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A disadvantage of the lead strains used in electron microscopy is the amount of time required for their preparation. One of the more stable and reliable stains, commonly called the lead citrate stain or Reynolds' stain (Reynolds, 1963), 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 communication reports the use of a commercially available lead citrate[1] to eliminate the preparatory steps of Reynolds' procedure. A 0.1 to 0.4 per cent concentration of this lead citrate in approximately 0.1 N sodium hydroxide forms a stain which provides contrast and delicacy equal to that of Reynolds' stain. The advantage of this mixture is its ease of preparation.

To make 10 ml of the stain, simply weigh out 0.01 to 0.04 grams of lead citrate, add it to 10 ml of singly distilled water in a screw-topped centrifuge tube, then drop in 0.1 ml of 10 N sodium hydroxide. Close the tube tightly and shake it vigorously until all the lead citrate is dissolved. The total operation takes less than 5 minutes.

[1] K & K Laboratories, Inc., Plainview, New York.

To prevent the formation of lead carbonate as a precipitate, avoid contaminating the staining solution with carbonate ions. Use only concentrated sodium hydroxide solutions or sodium hydroxide pellets in making the stain since these forms contain little sodium carbonate. Also, keep the tube sealed from atmospheric carbon dioxide. A lengthy exposure to the atmosphere must occur before precipitates render the stain useless.

Stain sections by placing them, while on supporting grids, either in or upon small quantities of the staining solution dropped on clean wax or glass. Staining time required for Epon or Araldite sections varies from 10 seconds to 5 minutes, depending upon the type of tissue and its previous treatment. Wash the stained sections in distilled water; a recommended method consists of twenty rapid dips of the grids into each of two or three vessels of distilled water. Air-dry them on filter paper before examining them in the electron microscope.

Staining times of 1 minute or less are adequate for most tissues and lessen the chance of the formation of stain precipitates. A longer time often overstains sections, reducing contrast between the cellular components. Double staining, by staining first with aqueous uranyl acetate then with lead citrate, requires only a few seconds in each solution. In this case, longer staining may result in coarse, ill-defined images of unit membranes at high magnifications.

If large particulates (100 mμ or larger) contaminate *otherwise clean sections*, discard the stain. If fine dots (3 to 15 mμ) appear in stained sections, but not in unstained ones, lower the concentration of lead citrate or shorten the staining time since the section is probably overstained. If the dots also are present in the unstained sections, they may be due to non-specific osmium tetroxide precipitation in the tissue.

Unexplained failures in staining are best dealt with by throwing out the stain and making a fresh solution. If failure is repeated, seek a new source of sodium hydroxide and purer water.

Usually when the stain is discarded some precipitate remains on the wall of the tube. To remove it put sodium hydroxide pellets in the tube, add distilled water, and boil gently. The same tube can be used repeatedly for preparing fresh solutions.

This stain has been used in this laboratory for approximately 1 year. All tissues so far examined, both vertebrate and invertebrate in origin, have been successfully stained by this procedure.

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BIBLIOGRAPHY

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