A Simple Staining Technique for Electron Microscopy with Lead-Uranyl Acetate

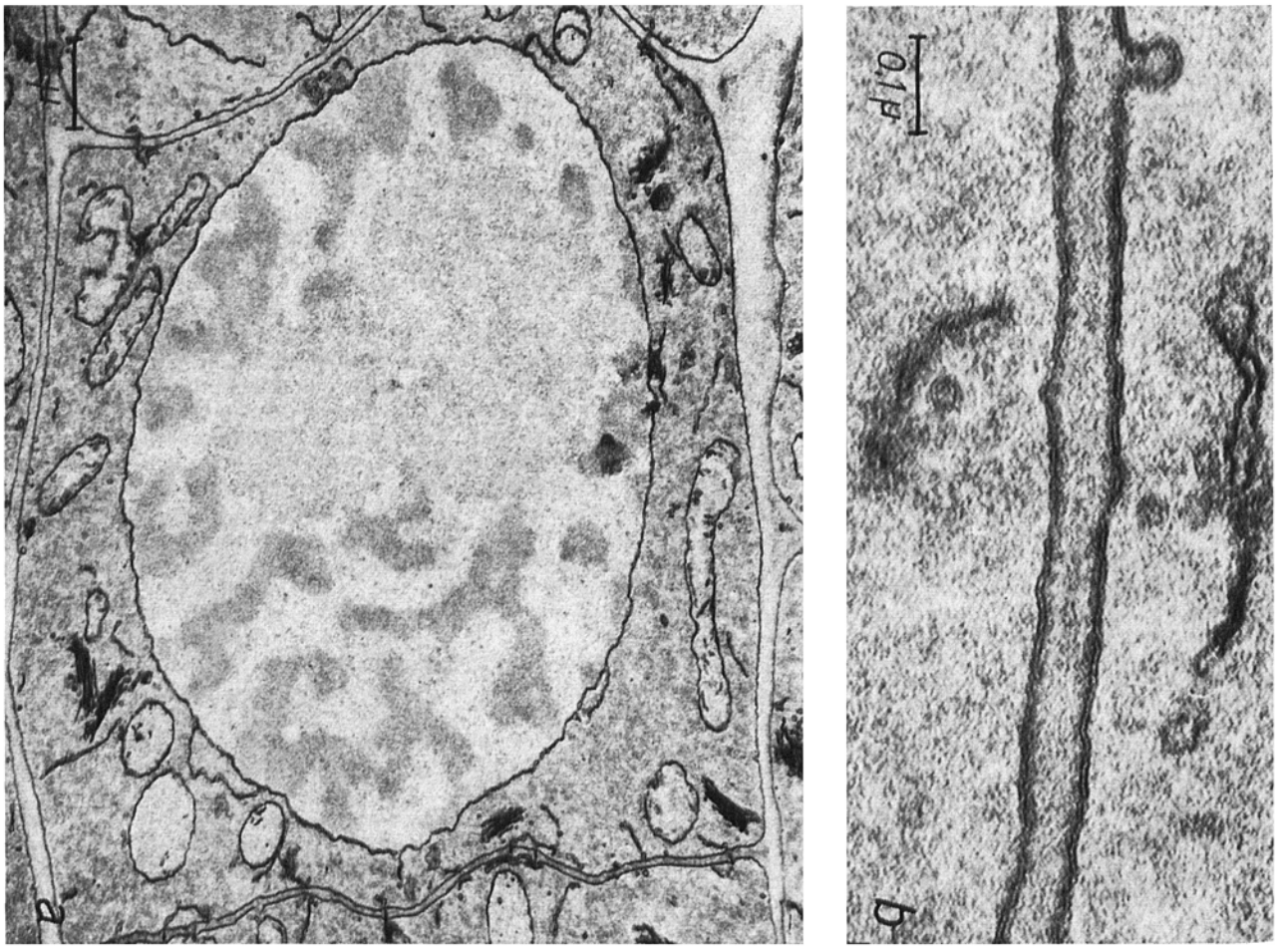
In electron microscopy the image is really no more than a magnified projection of the various densities proper to the components of the section. In order to achieve a differential increase of the densities proper to biological structures, which are not in themselves very opaque to electrons, the fixers themselves generally impregnate the various structures with heavy atoms, but the differential contrast thus achieved is not generally enough to give the picture sharp definition. A number of researchers[1-4] have tried to avoid this drawback by introducing impregnation with atoms of heavy metals after fixing, before embedding, and this has proved successful; with the further consequence that, to avoid the risk of distorting delicate biological structures by such impregnations, the material was protected by staining, after inclusion, applied to the ultrafine sections themselves[5-8].

For this, REYNOLDS[6] suggested a technique using lead citrate at high pH, which gave such good results that it has become one of the most commonly used techniques for producing the required contrast effect. But it has the same drawback as all other impregnation techniques applied to sections, although in a lesser degree, namely the frequent appearance of more or less intense contamination, which is difficult to avoid and, by a curious coincidence, generally most intense precisely in the areas of maximum interest.

In this paper, we give details of a contrast technique which we consider to possess many of the advantages of the above-mentioned technique, while it also has the additional advantage of avoiding contamination. It is based on enhancing the contrast presented by the object by impregnating it, after fixing, during the process of dehydration in acetone with a saturated solution of lead-uranyl acetate in acetone at 70%.

Material. 2 saturated solutions are prepared with excess reagent, one containing 25 cm³ of acetone at 70% and 5 g of neutral lead acetate, the other 25 cm³ of acetone at 70% and 2 g of uranyl acetate. The 2 solutions are mixed by being shaken together several times throughout 1 day,

1 J. R. G. BRADFIELD, Nature *173*, 184 (1954).
2 C. E. HALL, M. E. JAKUS and F. O. SCHMITT, J. appl. Phys. *16*, 459 (1945).
3 S. STRUGGER, Naturwissenschaften *15*, 357 (1956).
4 F. WASSERMANN and L. KUBOTA, J. biophys. biochem. Cytol. *2*, No. 4, suppl. 67 (1956).
5 A. J. DALTON and R. F. ZEIGEL, J. biophys. biochem. Cytol. *7*, 409 (1960).
6 E. S. REYNOLDS, J. biophys. biochem. Cytol. *17*, 208 (1963).
7 M. L. WATSON, J. biophys. biochem. Cytol. *4*, 475 (1958).
8 M. L. WATSON, J. biophys. biochem. Cytol. *4*, 727 (1958).



(a) Contrast with lead-uranyl acetate. Meristematic cell of *Phalaris canariensis* showing a similar contrast to that obtained by REYNOLD's technique. (b) In the 2 plasmalemma of this cell plate, the unit membrane can be seen without distortion of the fine structure.

thus producing an orange-coloured precipitate which is probably a lead uranyl complex together with a surplus of lead acetate.

Method. Germinating seeds of *Phalaris canariensis* were used, the root-tips being fixed in permanganate of potash at 2% in distilled water for 2 h at room temperature. The fixed material was then dehydrated in the acetone series up to that of 70%. At this stage the roots usually remain all night in a saturated solution of uranyl acetate in acetone at 70%, but we substituted lead uranyl for this solution and then continued the process of dehydration and inclusion in Durcupan ACM by the usual method.

Results and discussion. The pictures thus obtained (Figure) are both quantitatively and qualitatively on a par with those obtained by the use of REYNOLD's stain in point of contrast and definition. Since the impregnation is done without prolonging the normal process of dehydration and inclusion, and the sections can be observed as soon as they have been obtained, our method enables us to avoid a certain amount of handling and saves time.

One of the drawbacks of all lead solutions is that they age so quickly, with the consequent increased risk of contamination. In the present case we were able to observe that the lead uranyl solution presented no signs of alteration after 3 months, and examination of the sections from roots contrasted by this procedure showed no contamination of any kind. We think the absence of contamination is due to the fact that the piece itself acts as an ultrafilter, retaining any particles the solution may contain on its surface, and the contrast is effected by uranium and lead atoms forming a deposit upon the structures under study and avoiding such aggregations as give rise to the sort of particles that cause contamination.

Resumen. Mediante el empleo de una solución saturada de acetato de uranilo-plomo en acetona de 70%, durante el proceso convencional de deshidratación, se ha conseguido incrementar marcadamente el contraste electrónico en ápices radicales de *Phalaris canariensis*, sin riesgo alguno de contaminación o deformación de las estructuras.

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A Simple Method for Reduction of Non-Specific Fluorescence

The advantages afforded by the sensitivity and rapidity of immunofluorescence may be nullified by non-specific dye deposition[1,2]. This problem is particularly acute with exudate specimens of viral antigens where debris tends to intensify background fluorescence[3]. The empirically derived procedure presented here, utilizes methods similar to those reported from other laboratories but combines them in a specific sequence[4-7].

The following is a summary of 1 of 3 experiments utilizing vaccinia infected chorioallantoic membranes (CAM) and HeLa monolayers which demonstrated the necessary sequence of adsorption and gel filtration. Fluorescein isothiocyanate labeled vaccinia antibody was dialyzed and 1.0 ml aliquots stored at − 70 °C[7]. Immediately before use, 1.0 ml of the conjugate was thawed, adsorbed once with 100 mg of buffered-saline saturated rhesus monkey liver powder and centrifuged at 23,000 *g* for 30 min at 4 °C. The supernatant was then transferred to a Sephadex-25 (medium) column which measured about 0.6 · 10.0 cm. Collection of 1.5 ml of eluate was carried out with 0.02*M* phosphate buffer, pH 7.6. Other aliquots were treated as follows: (1) 2 adsorptions, (2) gel filtration alone, and (3) gel filtration followed by adsorption. All 4 aliquots were utilized simultaneously using standard washing and mounting procedures[7].

The Table presents staining intensities obtained with conjugates processed by different combinations of liver powder adsorption and gel filtration. Adsorption followed by gel filtration gave maximum staining intensity with infected cells where conjugate-antigen reaction was unblocked by negative serum. In addition the elimination of background staining resulted in the desired absence of fluorescence in uninfected cells or infected cell preparations pre-treated with vaccinia serum. The striking differences in staining are demonstrated by Figures (a), (b), and (c). All 3 photomicrographs are of vaccinia infected HeLa cells. Cells in (b) and (c) received a vaccinia serum

Immunofluorescence intensity with vaccinia infected CAM and HeLa cells

Conjugate treatment	Infected cells		Uninfected cells	
	Vaccinia serum	Negative serum	Vaccinia serum	Negative serum
I Adsorption and gel filtration	0	++++	0	0
II Gel filtration and adsorption	++	++++	++	++
III Gel filtration	++++	++++	++++	++++
IV 2 adsorptions	++	+++	++	++

(1) Method: direct staining. (2) Pre-conjugate serums: 1:4 dilutions of vaccinia and negative rabbit serums. (3) Conjugate: vaccinia rabbit globulin-isothiocyanate.

1 A. H. COONS, Rev. Microbiol. *8*, 333 (1954).
2 E. BEUTNER, Bact. Rev. *25*, 49 (1961).
3 M. SCHAEFFER, E. V. ORSI and D. WIDELOCK, Bact. Rev. *28*, 402 (1964).
4 W. GEORGE and K. W. WALTON, Nature *192*, 1188 (1961).
5 M. A. GORDON, M. R. EDWARDS and V. N. TOMPKINS, Proc. Soc. exp. Biol. Med. *109*, 96 (1962).
6 D. KIRSH and R. KISSLING, Bull. Wld Hlth Org. *29*, 126 (1963).
7 W. B. CHERRY, M. GOLDMAN, T. R. CARSKI and M. D. MOODY, Publ. Hlth Serv. Publs, Wash. *729*, 1 (1960).