IMPROVED RESOLUTION IN LIGHT MICROSCOPE RADIOAUTOGRAPHY

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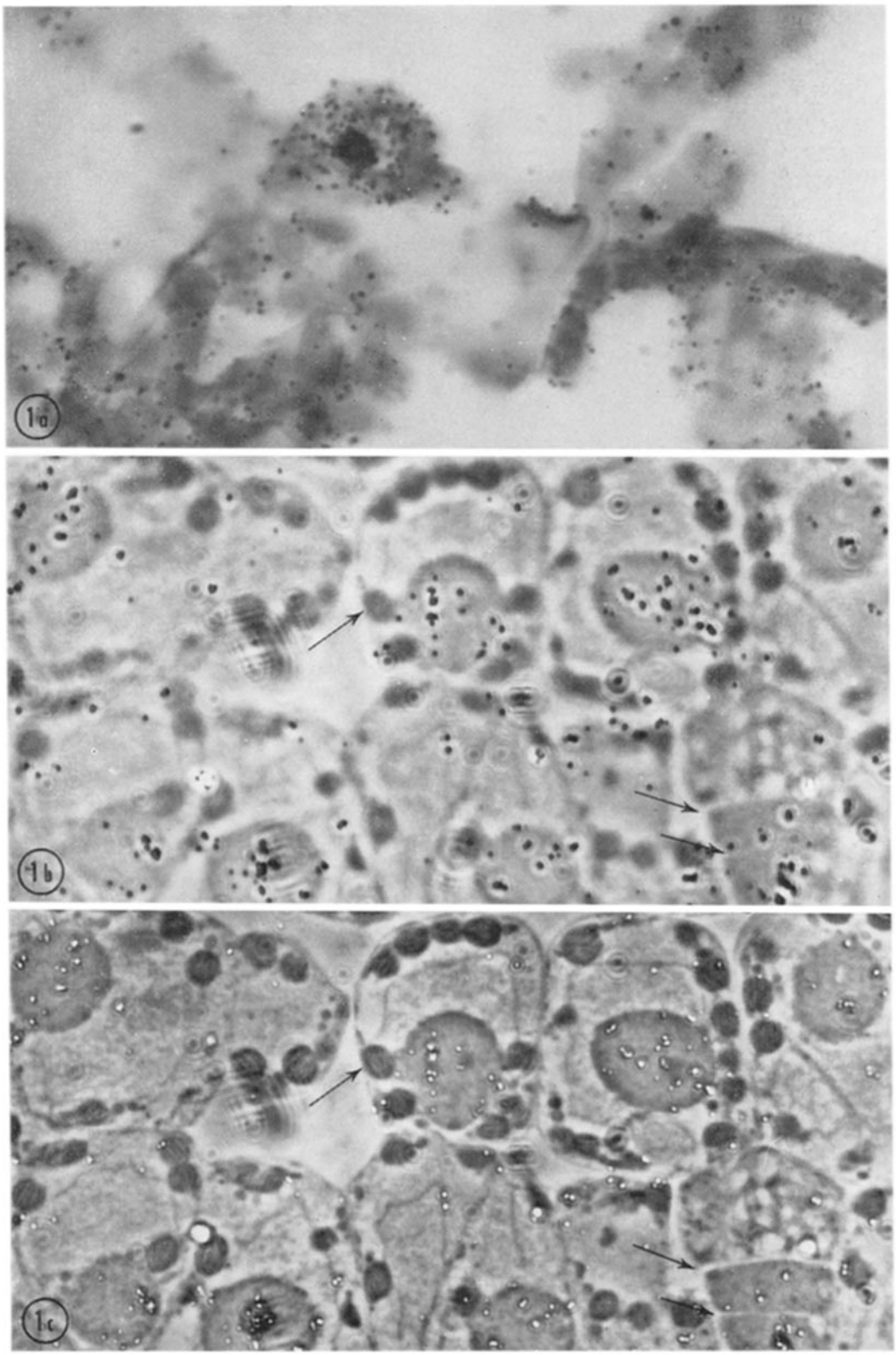
Photographic presentation of light microscope radioautograms is difficult since the silver grains and the sections lie in two different planes. At the high magnifications necessary for maximum resolution of fine grain emulsions, it is impossible to get both the grains and the section in focus. This causes little difficulty under the microscope since one can focus at will on each plane alternately in rapid succession. Photography obviously does not lend itself very well to this type of biplanar focusing, and the result is generally a compromise photomicrograph which shows neither plane in focus (Fig. 1 *a*).

In an attempt to improve on the photographic presentation of radioautograms, we have used a procedure which records the section in sharp focus. In these photomicrographs the silver grains are visualized as their overfocus, phase contrast image, and appear sharply outlined as bright dots. To one accustomed to the black grains

FIGURE 1 Radioautographs of tobacco leaf tissue labeled with uridine-^{3}H. (*a*) Paraffin section 5 μ thick, stained with chlorazol black. None of the cell organelles is clearly visible. There is also severe physical damage to the cells, and some of the cell walls have been flattened and moved, probably as a result of the emulsion drying down. (*b*) Araldite 6005 section 0.5 μ thick, phase contrast photograph with silver grains in focus. Note that nuclei are vague and cell outlines only partially visible. (*c*) Same section as *b*, but photographed with microscope focused on top of sections. Note the bright silver grain spots corresponding precisely to the black grains in *b*. Careful comparison of *b* and *c* shows that the resolution of grains is better in *c*. The arrow in the center of *b* shows a chloroplast as a fuzzy gray body, whereas this same chloroplast even shows an indication of lamellar structure in *c*. The two arrows in the lower right point to two separate cells which could not be resolved in *b*. Membranes are also clearly visible in *c*. All figures × 2200.



usually seen, this may be confusing at first, but the higher resolution possible in such photographs more than compensates for this inconvenience.

METHODS

Radioactively labeled (uridine-^{3}H) plant tissue was prepared for sectioning according to standard methods used in thin sectioning. The tissues have regularly been fixed in Dalton's buffered osmium tetroxide (2), dehydrated in a graded series of alcohols, and embedded in Araldite 6005 (Ciba Products, Fairlawn, N.J.). For determining the optimal section thickness for photographic reproduction, 0.5-, 0.75-, 1.0-, and 1.5-μ sections were cut and floated on a drop of distilled water on a clean glass microscope slide. The slide was warmed slightly over an alcohol flame, so that we could flatten and expand the sections, and then it was allowed to dry. The location of the sections on the slide was marked on the reverse side by the inscription, with a diamond marking pencil, of a circle around the sections. This made it possible to locate each droplet of sections at any time.

The individual slides were dipped in a 1:1 mixture of Ilford L4 nuclear emulsion (Ilford Limited, England) which had been melted and allowed to cool to room temperature. The slides were then dried and exposed over silica gel in sealed cans. Development was as recommended by Caro (1); however, the developed and dried slides were mounted with a No. 1 cover slip on a drop of Harleco Synthetic Resin instead of water or glycerin.

A Zeiss WL microscope equipped with phase optics, a planachromatic 100× oil immersion lens, and a green filter was used to examine the slides. Photomicrographs were made with the Zeiss 35-mm attachment camera. All pictures were taken on high contrast copy film (Eastman Kodak), which has extremely fine grain. Development was in D19 (Eastman Kodak) for 4 min.

All four section thicknesses provided excellent radioautographic images. The 0.5-μ and 0.75-μ sections were of low visual contrast and, in that sense, less desirable for microscopic viewing than the 1.0-μ and 1.5-μ sections, which were of higher contrast.

RESULTS

From the standpoint of photographic reproduction by the usual methods, none of these sections was satisfactory since very little cell detail could be seen when the exposed silver grains were in sharp focus (Fig. 1 *b*). Greatly improved images were observed when the microscope was focused on the very top of the section. Under these conditions, the silver grains were overfocused, and appeared as bright dots with the section itself in quite good focus (Fig. 1 *c*). Under the photographic conditions employed, the 0.5-μ and 0.75-μ sections provided the best photographs (Fig. 1 *c*). The 1.0-μ and 1.5-μ sections yielded a photographic image of such high contrast that it lacked detail even when printed on No. 1 or 2 paper.

This method of photomicrography requires careful attention to microscopic techniques if high resolution is to be achieved. Proper illumination and phase alignment are absolutely essential, and in their absence very little can be seen. This method has been very successful when low energy tritium was used. Preliminary results with ^{125}I have been somewhat less satisfactory probably because the greater decay energy of this isotope resulted in the grains' appearing at greater distances from the section. Presumably similar, less desirable results might also occur with ^{14}C and ^{32}P. Therefore, it was difficult to get both the section and bright grain spots sharp as was done in Fig. 1 *c*, although acceptable radioautograms can still be readily obtained. In addition, the flat surface of the plastic section makes grain counting from photographs easier since all grains are essentially in the same plane (Fig. 1 *c*). Contrast this with the 5-μ paraffin section which has been freed of wax before coating with emulsion (Fig. 1 *a*). Here the silver grains appear at all levels since the surface of the section is not flat.

Removal of gelatin with 0.1 N NaOH, as is routinely done in electron microscope radioautography, did not improve the images for light microscopy. Staining with toluidine blue (3) was not satisfactory when the gelatin was present, and when staining was done after removal of gelatin there was frequently an almost total loss of silver grains.

Undoubtedly the use of other films, developers, and printing papers would enable one to get good photographs from the thicker sections. There are, however, several compelling reasons for using sections as thin as possible. Caro (1) has shown that there is a considerable increase in resolution as the thickness of the section and emulsion decreases. In these 0.5-μ sections, any point in the section is within "range" of the emulsion for tritium β particles, and the problem of superimposition so difficult in thicker sections is eliminated. Resolution is also improved by use of the permanent mounting media which requires dry emulsions and results in a decrease in thickness of the gelatin layer. Furthermore, we routinely make light microscope radioautograms in preparation for electron microscope radioautography.

This procedure provides valuable information on the quality of the embedded material and permits accurate determination of the exposure time necessary for electron microscope radioautography. By using several sections only 0.5 μ thick for light microscope radioautography, we have ample material left for further electron microscope studies on the same cells.

SUMMARY

A method for preparing light microscope radioautograms which permits clear visualization of both silver grains and cell contents is described. Radioautograms were prepared of 0.5–0.75-μ plastic sections using a thin layer of emulsion. Photographs were made with phase optics and the silver grains were overfocused. Under these conditions, the silver grains appear as bright spots and the section is in sharp focus.

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