The Use of Potassium Permanganate as an Electron-Dense Stain for Sections of Tissue Embedded in Epoxy Resin.*

By A. M. Lawn.‡ (*From the Department of Anatomy, Washington University School of Medicine, St. Louis.*)

Potassium permanganate has become an established fixative in electron microscopy (Luft, 1956) and has special value in the study of cell membranes. During an investigation of methods of increasing the contrast in the electron microscope of tissues embedded in epoxy resin,[1] a solution of potassium permanganate was used to treat the sections, using a technique similar to that of Watson (1958). Potassium permanganate was found to produce a marked increase in contrast, but this was usually accompanied by a granular precipitate of electron-opaque material on the surface of the section. It was found that a weak solution of citric acid could be used to remove this precipitate without seriously reducing the contrast of the section. The following method is suggested as a starting point for further improvement.

Staining Solution.—Dissolve 1 gram of potassium permanganate crystals in 100 ml. of distilled water. Do not filter. Allow to stand at least 24 hours before use.

Staining Procedure.—1. Fill a small vessel with staining solution removed with a pipette from beneath the surface film in the stock bottle.

2. Sweep the surface of the staining solution clean with the edge of a microscope slide coated with paraffin wax.

3. Float thin sections (without removal of the epoxy resin) mounted on collodion-coated copper grids, section side down, on the surface of the staining solution immediately after the surface has been cleaned. Stain for 15 minutes to 2 hours.

4. Rinse in running distilled water for a few seconds.

5. Immerse and agitate in a solution of one drop of 5 per cent citric acid in 1 ml. distilled water for 30 seconds to 1 minute.

6. Rinse in distilled water.

7. Dry by placing grids section side up on filter paper.

Even with the precautions used in this procedure, precipitation may occur, but it does not usually limit the usefulness of the section. The time of staining required varies with fixation (both Dalton's and Palade's fixative have given good results), the degree of contrast desired, the type of epoxy resin, and other unknown factors, and must be determined by trial.

Potassium permanganate appears to stain nearly all of the known components of tissues when used in this manner, and, therefore, the resulting micrographs lack the clarity of micrographs of tissues fixed in permanganate solutions in which cell membranes are selectively stained. The citric acid has a tendency to cause tissue damage, for example swelling of collagen fibres. Because of the high contrast achieved, any defects of fixation are exaggerated.

In spite of these disadvantages, the method appears to warrant further trials. It overcomes the difficulties that result from the inherent low contrast of sections of osmic-fixed tissues embedded in epoxy resin, resulting in contrast as good as or better than unstained methacrylate-embedded sections of equal thickness, so that focusing is greatly facilitated, resolution improved, and the effect of photographic grain diminished. This is accomplished without special fixation of the tissues and without staining a whole block of tissue, enabling other sections from the same block to be examined unstained or after staining by other methods.

In addition, certain structures are emphasized; for instance, tonofibrils, basement membranes, terminal bars, and desmosomes. Preliminary trials indicate that this staining method is also useful for staining sections of tissues embedded in methacrylate.

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‡ On leave of absence from the Department of Physiology, Royal Veterinary College, University of London, England.

[1] The epoxy resin used was "araldite" supplied partly by the New York Society of Electron Microscopists and partly by Aero Research Ltd., England.

References

Luft, J. H., Permanganate—A new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.

Watson, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.

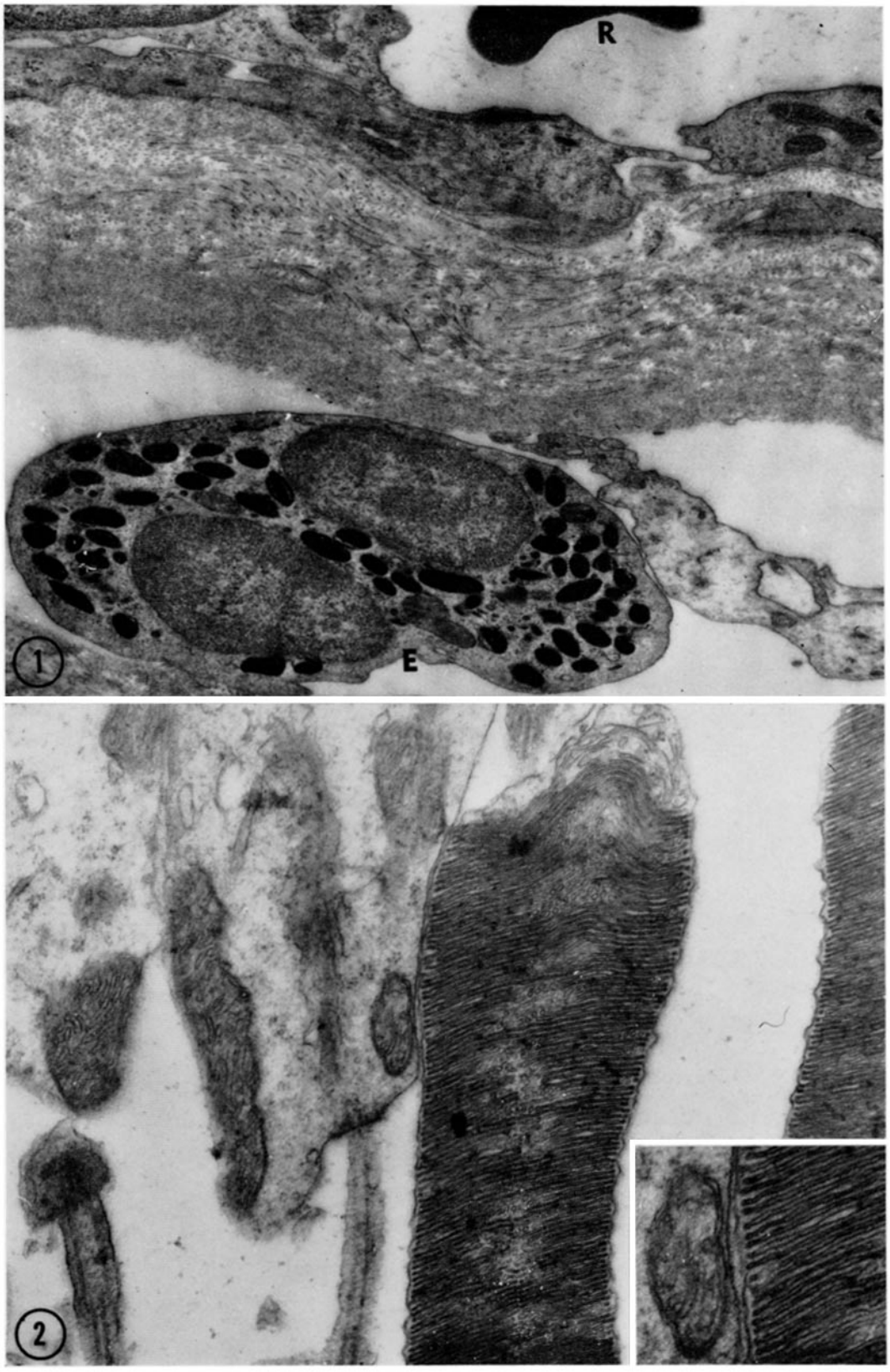
Explanation of Plates

All the figures are of thin sections cut from material fixed with osmium tetroxide solutions and embedded in epoxy resin. The thin sections were stained with a solution of potassium permanganate using the method described in the text.

Plate 103

Fig. 1. Part of a blood vessel and tissue space from the stomach of a rat. The red blood cell (*R*), the granules of the eosinophile leukocyte (*E*), and the collagen fibres (with associated fine fibrils) of the sheath of the vessel are all stained. × 11,000.

Fig. 2. Retinal rods of an adult mouse. Membranes of the cell surface and lamellae of the rods are well shown. × 48,000. (The author is indebted to Dr. A. I. Cohen for permission to publish this micrograph which is part of a study of the adult and embryonic eye.)



(Lawn: Potassium permanganate as electron-dense stain)

PLATE 104

FIG. 3. A small region from the brain stem of a mouse. At the arrow an increase in density is seen where a cell process filled with "synaptic" vesicles is in close contact with another cell process. Other less well marked contact areas can be seen. × 9,200.

FIG. 4. This portion of the cell body of a neuron from the brain stem of a mouse, shows the Golgi zone (*G*), mitochondria (*M*), and a microbody (*MB*). Membranes with attached particles are prominent throughout the cytoplasm. × 32,000.

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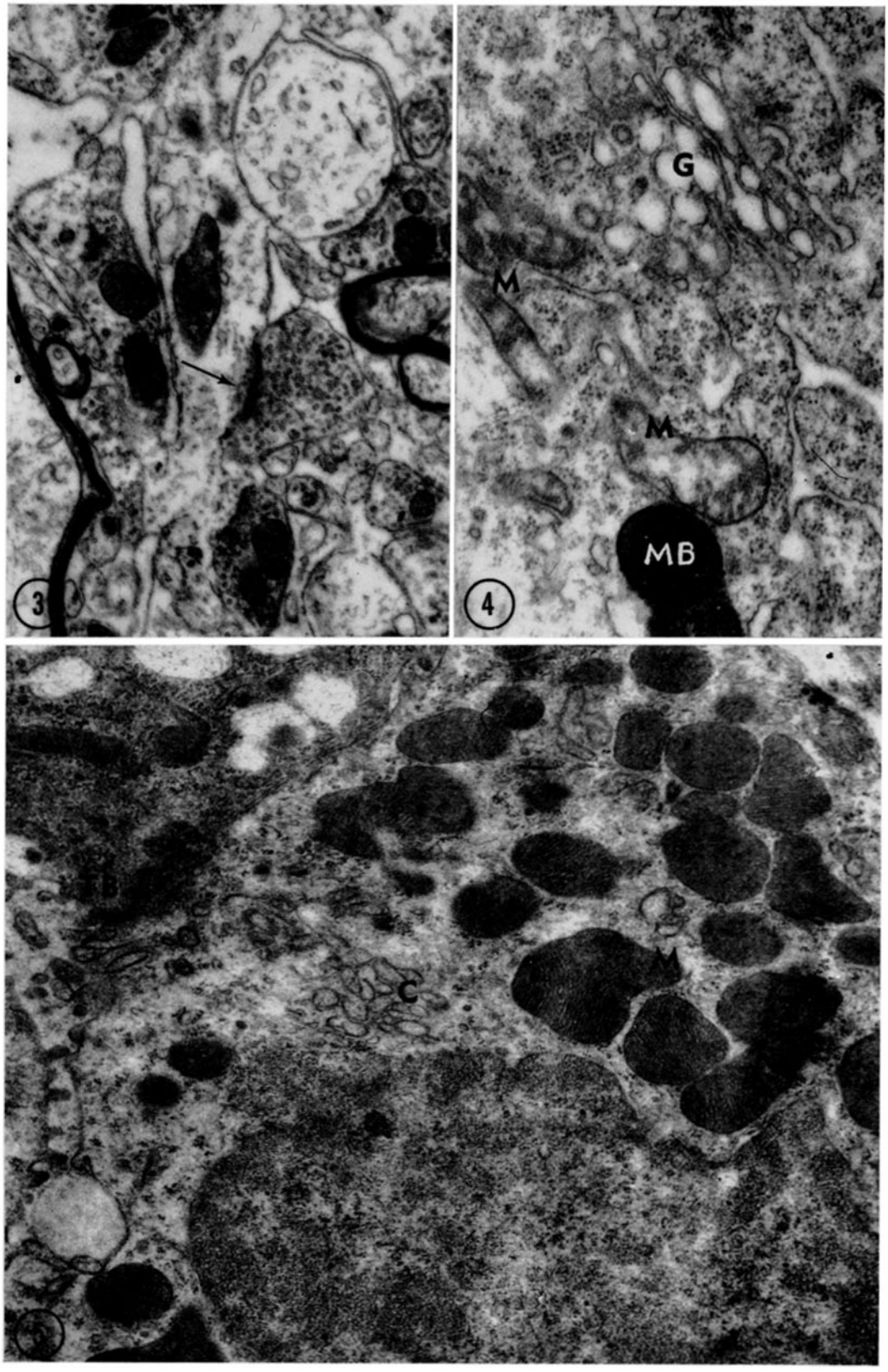
FIG. 5. A section through part of a parietal cell from the stomach of a rat. The mitochondria (*M*) with their dense matrix and closely packed cristae contrast with the pale cytoplasm. The surface membranes, particularly of intracellular canaliculi (*C*), can easily be discerned and an increase in density (*TB*) is associated wth the junction of the parietal cell and a mucous epithelial cell at upper right. × 14,000.



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