Permanganate—A New Fixative for Electron Microscopy.*

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During the early phases of tissue sectioning for electron microscopy, virtually all of the standard cytological fixatives were tried. Of these, osmium tetroxide appeared to give the best preservation, and this was further improved by buffering the fixative solution at a slightly alkaline pH (Palade (1)). A variant developed by Dalton (2) contains OsO_4 in a chromate-dichromate system. Formalin has been used for special purposes for electron microscopy, and a fixative without OsO_4 has been described by Low (3), consisting of a chromic acid–formaldehyde mixture.

Potassium permanganate, or more specifically the permanganate ion, has occasionally been used as a fixative or stain in light microscopy (4). It provides remarkable preservation of many cell components at the electron microscope level, as the following plates illustrate. The tissue fine structure looks similar to that following osmium treatment, thus rendering less likely the criticism that electron microscopists are merely seeing artifacts of osmium fixation. Besides preserving fine structure, permanganate fixation enhances tissue density and contrast. This feature is of interest because of the low atomic weight of manganese (54.9) in comparison to the high atomic weight of osmium (190.8). It would seem that explanations of tissue contrast must consider other factors besides the atomic weight or number of the fixative components.

Permanganate appears to fix membrane systems first, (within 15 to 30 minutes), allowing the remaining cellular components either to leach out during dehydration, or to be volatilized in the electron beam as reported by Morgan *et al.* (5). Longer fixation (1 to 12 hours) retains other structures such as nuclear chromatin, the nucleolus, and the matrix of mitochondria. One hour fixation retains enough nuclear material to give a moderate Feulgen reaction in sections 2μ thick. Sodium permanganate appears to offer no advantage over the potassium salt, nor does the addition of OsO_4 to the permanganate. The actual details of the method are as follows:

A stock solution of reagent grade potassium permanganate ($KMnO_4$) is made up in distilled water to 1.2 per cent *w/v* and stored in the refrigerator. It is best to keep the stock solution in a well filled, glass-stoppered bottle since $KMnO_4$ solutions are slowly reduced on contact with air. This solution replaces the 2 per cent OsO_4 stock solution used to prepare the usual 1 per cent buffered OsO_4 (Palade (1)). For use, equal volumes of the permanganate stock solution are mixed with the veronal-acetate buffer, giving a final concentration of 0.6 per cent $KMnO_4$ at the pH selected—generally pH 7.4–7.6. Within the range tested, namely from 0.5 to

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1.2 per cent, the concentration of $KMnO_4$ does not appear to be critical. The buffered $KMnO_4$ solution is cooled in cracked ice, and 1 or 2 mm. cubes of tissue are fixed at O°C. for 15 minutes to 12 hours. Tissue fixed in this manner but at room temperature seems to be badly damaged; the effect of intermediate temperatures has not been established. At the end of fixation, the tissue is rinsed for several minutes in cold (0–5°C.) 25 per cent ethanol, and allowed to warm to room temperature in fresh 25 per cent ethanol, (15 to 20 minutes). The blocks are then dehydrated through graded alcohols as usual, and taken into *n*-butyl methacrylate in which they are embedded. The tissue seems to be more difficult to cut with glass knives than equivalent osmium-fixed material.

It is unlikely that $KMnO_4$ will replace OsO_4 as a general purpose fixative. Tissue structure shows a somewhat granular texture, the 150 A cytoplasmic granules which have a high RNA content are absent, and mitochondria appear swollen. Membranes, however, are remarkably distinct. Plasma membranes of cells are exceptionally well defined, for example in the brush border of renal convoluted tubular epithelium (Fig. 2) and the C fibers of peripheral nerve (Fig. 3). The paired membranes surrounding mitochondria, and the cristae projecting from the inner membrane are readily apparent in Figs. 1 and 2. The high contrast of the membranes reveals nuclear pores in oblique sections of nuclear envelope (Fig. 1). The layers of the myelin sheath of peripheral nerve are well preserved, including the middle layer (Fig. 4). The vesicles characteristic of synapses are similarly clearly differentiated after permanganate fixation (Fig. 5). Granules in liver, which are probably glycogen, are preserved better than with osmium, and take the form of cytoplasmic rosettes or clusters, composed of small (100 to 150 A) globular elements (Fig. 1). Permanganate destroys the myofilaments of striated muscle, but the sarcoplasmic reticulum is clearly revealed in the interfibrillar spaces where it appears regularly disposed in relation to the A and I bands of the myofibril (Fig. 8). In the pancreas the delicate membrane surrounding the zymogen granules, and the conspicuous parallel array of endoplasmic reticulum are also well defined (Fig. 6). High resolution micrographs reveal a lace-like or reticular structure to these membranes (Fig. 7), in contrast to the homogeneous membranes seen after osmium fixation.

Permanganate fixation seems to demonstrate membrane systems with special clarity. It seems almost specific for the endoplasmic reticulum in its broad aspects (Porter (6)). It reveals a reticular structure in these membranes, which may be artifact, or may have some basis in reality. Permanganate is insoluble in oils and fats, in contrast to the high solubility and reactivity of OsO_4 in these materials. This feature may contribute to the further understanding of tissue fine structure in terms of the distribution of lipides and proteins.

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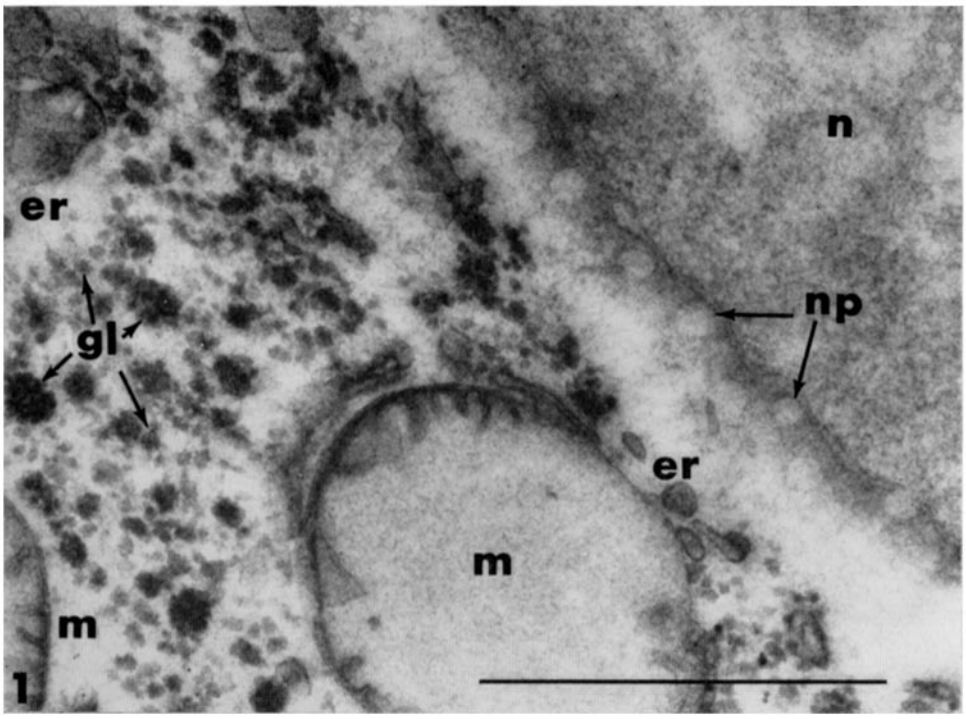
Explanation of Plates

The solid line represents one micron, unless otherwise noted.

Plate 223

Fig. 1. Electron micrograph of a portion of a mouse liver cell fixed 12 hours at 0°C. in 0.5 per cent $KMnO_4$ at pH 7.4. Mitochondria are present at *m*, and membranous and vesicular elements of the endoplasmic reticulum at *er*. A small portion of the nucleus is visible at *n*. The nuclear membrane is cut obliquely and reveals many nuclear pores (*np*). Material which probably is glycogen (*gl*) is apparent as rosettes and clusters of smaller particles which measure about 100 to 150 A in diameter. Taken with Siemens Elmiskop 1, total magnification 54,000.

Fig. 2. Electron micrograph of frog kidney illustrating the brush border of the epithelium of the proximal convoluted tubules. The tissue was fixed 1½ hours at 0°C. in 0.6 per cent $KMnO_4$ at pH 7.5. Mitochondria with cristae are present at *m*, and a portion of the nucleus at *n*. The brush border (*bb*) is composed of numerous projections from the cell surface extending into the lumen of the tubule at *l*. Vesicular and tubular elements of the endoplasmic reticulum (*er*) are present throughout the cytoplasm. Micrograph taken with RCA-EMU-2E, total magnification 12,000.



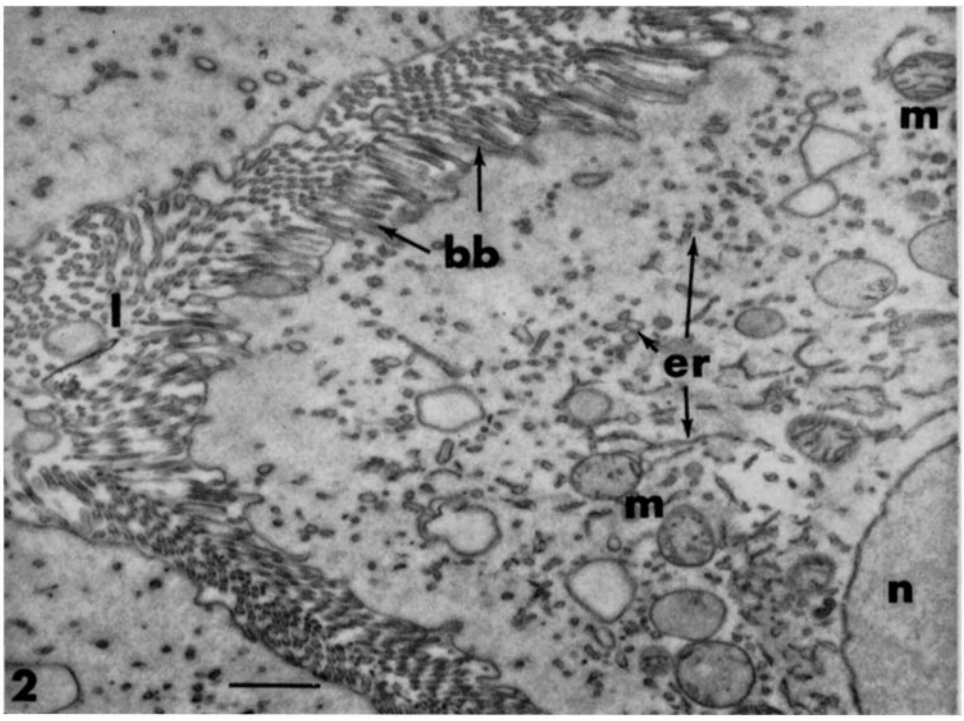


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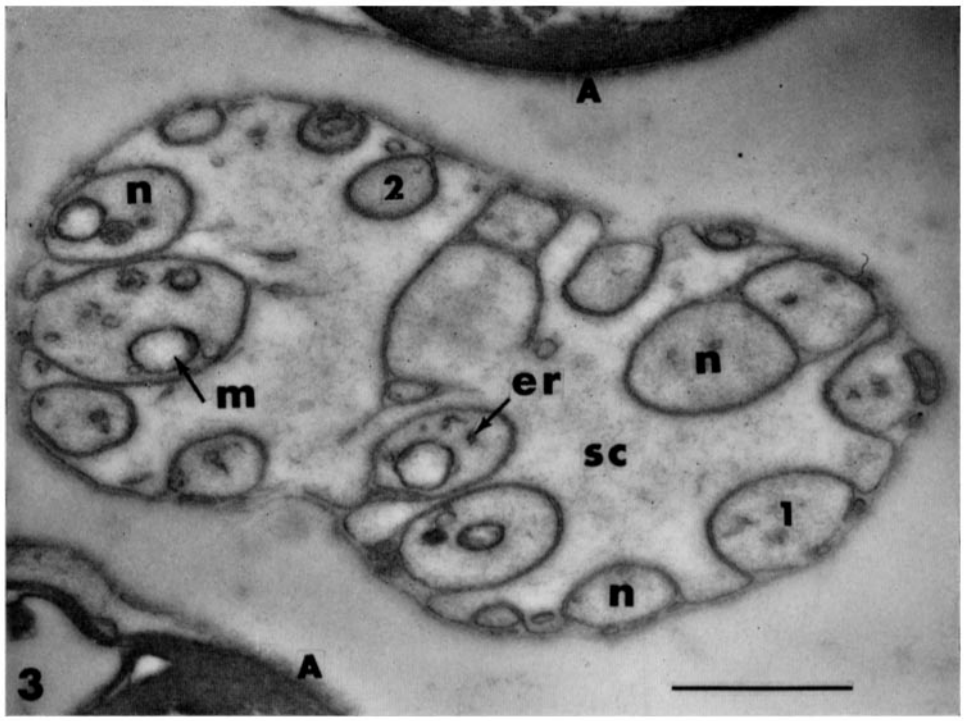
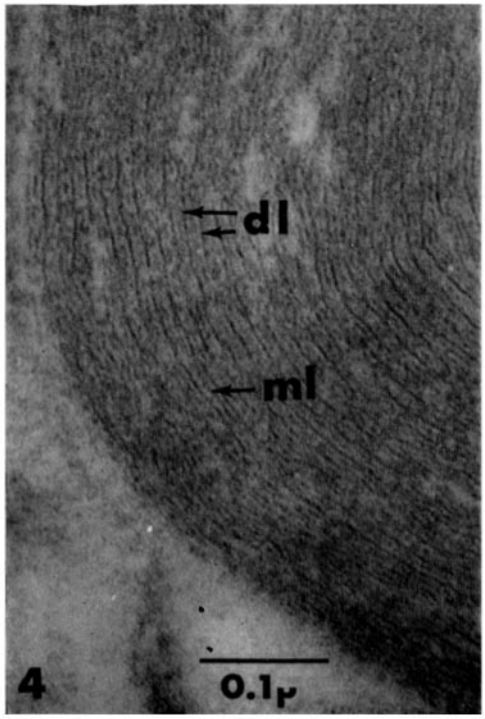
PLATE 224

FIG. 3. Electron micrograph of a cross-section of a bundle of C fibers in mouse sciatic nerve. Tissue was fixed 45 minutes at 0°C. in 0.6 per cent $KMnO_4$, at pH 7.4. The numerous nerve fibers (*n*) are embedded at the surface of the cytoplasm of the Schwann cell (*sc*). It is possible to trace the invaginated Schwann cell membrane partially (*1*) or almost completely (*2*) surrounding the axoplasm with its complete cell membrane. Swollen mitochondria (*m*) and vesicular components of the endoplasmic reticulum (*er*) are present in the neural elements. Myelin layers of two accompanying A fibers are seen at *A*. Micrograph taken with RCA-EMU-2A, total magnification 24,000.

FIG. 4. Electron micrograph of the myelin sheath of the A fibers in the same section as Fig. 3. The layers of the sheath are well preserved, spaced 115 A apart. These dense layers (*dl*) appear to be about 20 A wide, whereas the less dense middle layer (*ml*) between these appears to be about twice this thickness in many places. Taken with the Elmiskop 1 at a total magnification of 170,000.

FIG. 5. Electron micrograph of electric tissue of the electric eel, showing a nerve (*n*) making synaptic contact with the electroplaque (*el*). Tissue fixed 1½ hours at 0°C. in 0.5 per cent $KMnO_4$ at pH 7.3. Synaptic vesicles (*sv*) are seen in the nerve cytoplasm and near the site of synaptic contact (*x*). Micrograph taken with Elmiskop 1, total magnification 24,000.





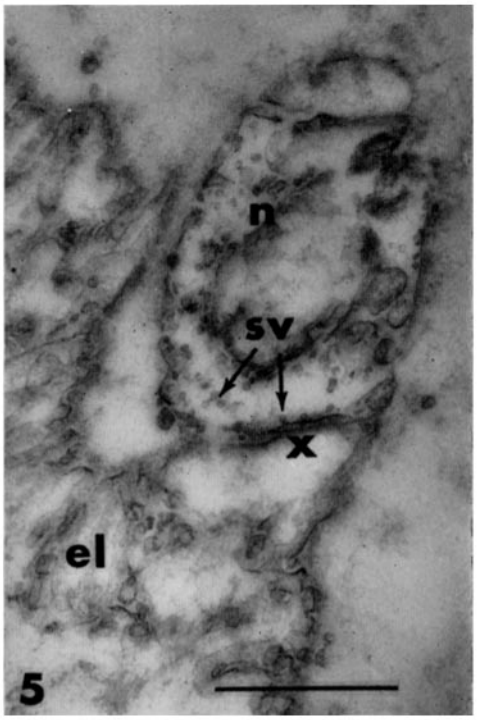


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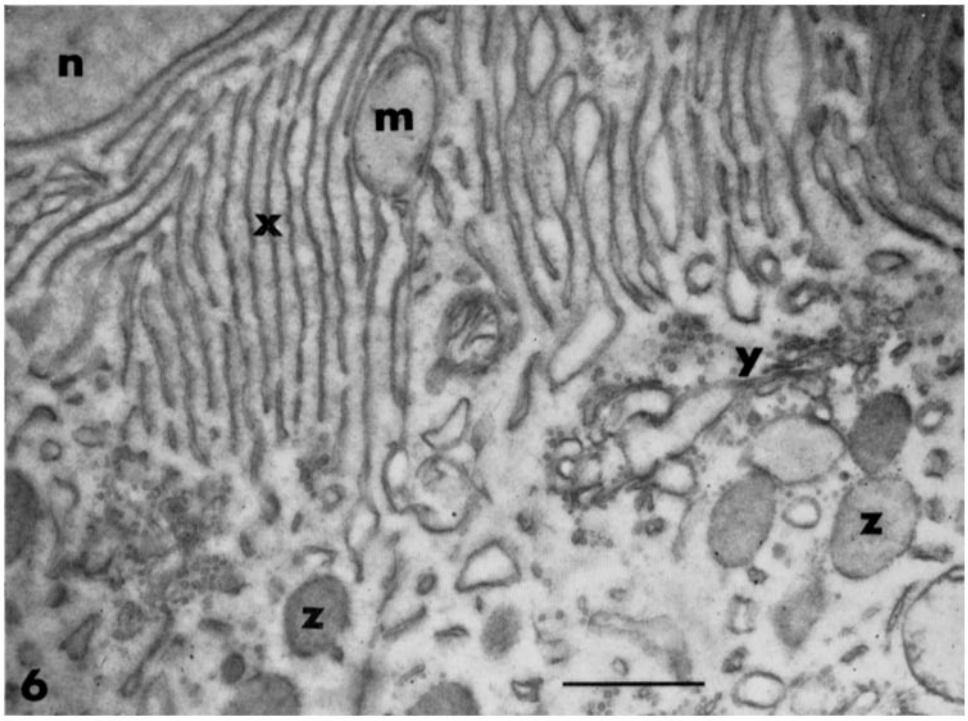
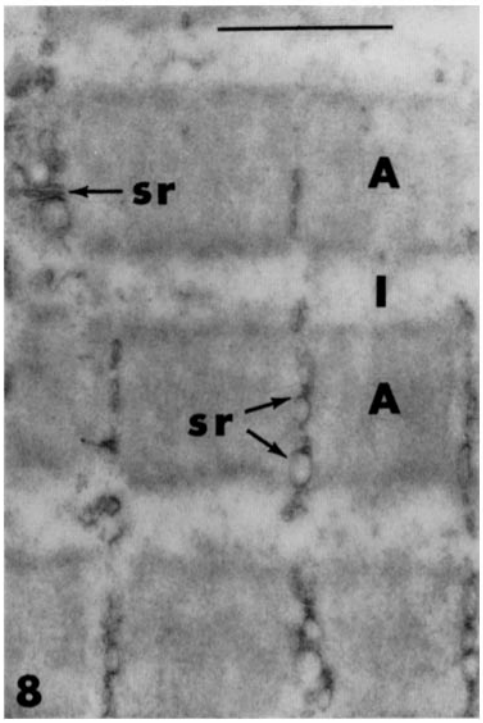
FIG. 6. Electron micrograph of a part of a cell from the exocrine pancreas of a mouse. Tissue fixed 45 minutes at 0°C. in 0.6 per cent $KMnO_4$, pH 7.4. A portion of the nucleus is present at *n*, and mitochondria at *m*. The endoplasmic reticulum is present in the familiar parallel cisternae at *x*, and as the tubular and vesicular forms of the Golgi apparatus at *y*. Zymogen granules (*z*) possess a delicate membrane enclosing them. Micrograph taken with RCA-EMU-2A, total magnification 18,700.

FIG. 7. Electron micrograph of the parallel sheets of endoplasmic reticulum of pancreas similar to Fig. 6, but fixed 60 minutes. Self-supporting thin section over holes in a carbon film. The granular or reticular appearance of the membranes is apparent. At the thinnest points, (presumably in cross-section), the membranes measure about 30 A in thickness. The 150 A, RNA-rich granules are absent. Taken with Elmiskop 1, total magnification 170,000.

FIG. 8. Electron micrograph of striated muscle from mouse diaphragm. Tissue fixed 1 hour at 0°C. in 0.6 per cent $KMnO_4$ at pH 7.4. The myofibrils run vertically with A bands and I bands labelled as such. Z bands are absent, and the continuity of myofilaments is lost. The sarcoplasmic reticulum (*sr*), however, is clearly present in its usual form, but is much more apparent because of the accentuated contrast. Micrograph taken with Elmiskop 1 at 23,000 total magnification.





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