SODIUM PERMANGANATE FIXATION FOR ELECTRON MICROSCOPY

BRUCE K. WETZEL. From the Department of Biology, Harvard University, Cambridge, and the Department of Anatomy, Cornell University Medical College, New York

Heightened interest in the cytology of certain free-living flatworms has been evident in the literature throughout the past year (5, 6, 10–12). The variety of problems dealt with by various investigators reflects the abundance of curious cellular specialization found in this group of animals. Moreover, the suitability of the Turbellarians for the study of regenerative processes is well recognized. Difficulties encountered in achieving satisfactory preservation of fine structure detail in *Dugesia tigrina*, a fresh-water Turbellarian, have led to a re-examination of several of the parameters involved in standard methods of preparation. During the course of these investigations, several of the more commonly used fixing agents have been tried with varied success. These include osmium tetroxide (9), chrome-osmium (2), buffered formalin, and potassium permanganate (7). Of these fixing agents, osmium tetroxide has yielded acceptable results, while potassium permanganate fixation has shown especially good preservation of all membranes except the cytoplasmic membrane.

Sodium permanganate as a fixative for electron microscopy has been previously mentioned, but without recommendation (7). A summary of the reasoning leading to its present use may be of interest.

It is noted that careful maintenance of a delicate balance among sodium, potassium, calcium, and to some extent magnesium ions in the immediate environment of a living cell is necessary to the maintenance of cell function and structure. Disturbance of this ion balance can seriously disrupt a cell, though the tonicity of the solution is kept constant.

The use of 1 per cent sodium permanganate as a fixing agent augments the concentration of sodium ion in the fixative by a value (51 mM/l.) well below the sodium ion concentrations generally reported for the body fluids of most organisms. Consequently, the ion balance of the fixative can be readily adjusted to approximate the ion balance of the cell's normal environment. When 1 per cent potassium permanganate is used as a fixative, however, the concentration of potassium ion contributed by the fixing agent alone (63 mM/l.) exceeds the values generally reported for potassium ion concentration in the body fluids of most organisms. This discrepancy in ion balance between the fixing fluid and the cell's normal environment might interfere with optimum fixation of the cell, perhaps more markedly in some tissues than in others.

The rationale employed in this investigation assumes that an approximation of the physiological extracellular conditions of a tissue is advantageous in formulating a successful fixing fluid. Such an assumption can only gain validity through a meticulous and exhaustive analysis of the prerequisites and mechanisms of satisfactory tissue preservation. Fixation is indeed so inadequately understood that the cytologist has little basis for choosing the extracellular environment over the intracellular one as a guide for formulating his fixative. Even the degree to which a fixing fluid need be "physiological" is still in question. Certainly a general evaluation of the role of ion balance in fixation cannot be drawn from the above-mentioned empirical approach to a particular problem. Yet, the results of this investigation would seem to merit careful consideration.

MATERIALS AND METHODS

The fixing fluid used for the tissue illustrated in this paper is essentially one devised by Michaelis (8) and developed for electron microscopy by Palade (9). It was prepared using a 2 per cent aqueous stock solution (*w/v*) of sodium permanganate,[1] or of potassium permanganate, in place of the osmium tetroxide stock solution recommended by the above-mentioned

[1] Obtainable from Fisher Scientific Company, Fair Lawn, New Jersey, in "purified" grade.

authors. The fixing fluid thus contained a final concentration of 1 per cent sodium permanganate, or 1 per cent potassium permanganate, respectively. The pH of the fixative was adjusted to a value of 6.0, which seems to be optimum for this tissue.

Living specimens of *Dugesia tigrina*, collected several days previously from a local stream, were immersed in chilled fixative and were there rapidly dissected into pieces approximately 4 mm. × 4 mm. × 2 mm. The tissue was fixed for 2 hours at a temperature of 1–2°C. The tissue was then washed in 3 changes of tap water over a period of 15 minutes, and subsequently dehydrated through a graded ethanol series. The dehydration schedule consisted of single ½ hour changes of 70 per cent, 95 per cent, and absolute ethanol, followed by 1 hour in a fresh change of absolute ethanol. Embedding in Araldite epoxy resin (4) was carried out with slight modifications (14) of the original methods. English Araldite resin M (Ciba) and English dodecenyl succinic anhydride (Ciba) were used along with dibutylphthalate and accelerator B secured from the New York Society of Electron Microscopists.

Sections were cut with a Porter-Blum ultramicrotome equipped with a glass knife, and collected from the surface of a 40 per cent acetone solution on etched 200-mesh copper grids coated with a supportive collodion film. The sections were then stained with potassium permanganate according to the method of Lawn (6). Lead acetate (3) and uranyl acetate (13) staining methods were also tried, but with little success. Sections were examined in an RCA EMU2-D electron microscope equipped with a 0.015 inch Canalco externally centerable platinum condenser aperture, and a 45 μ copper aperture in the standard objective pole piece. The micrographs were taken at magnifications of 4,200 and 5,100 diameters, and photographically enlarged to the final magnifications indicated in the legends.

RESULTS AND DISCUSSION

Luft (7) has pointed out that the effective fixing agent in either sodium or potassium permanganate fixatives is most probably the permanganate ion. Bradbury and Meek (1) have recently stated that potassium permanganate does not act as a fixative at all. Regardless of which view is correct, planarian tissues "treated" with sodium and potassium permanganates appear quite similar. The relative electron-scattering properties of various tissue components are nearly identical when the two fixatives are compared. Nuclei, mitochondria, muscle fibers, nerve fibers, gut cells, mucus-secreting cells, and a great variety of additional distinct morphological cell types found in the parenchyma of the planarian can be closely compared in these respects, irrespective of the cation introduced by the fixing agent.

Contrasting sodium and potassium permanganate fixations, slight differences, quite likely dependent on the fixative, may be observed generally in the cytoplasm and the nucleoplasm of the cells. These differences appear in the micrographs as variations in the "texture" of certain tissue components as well as in relative electron density. Whether such variation is entirely dependent on the fixative or represents physiological variation among different tissue components, should be resolved by the thorough study of the tissues of this organism now in progress.

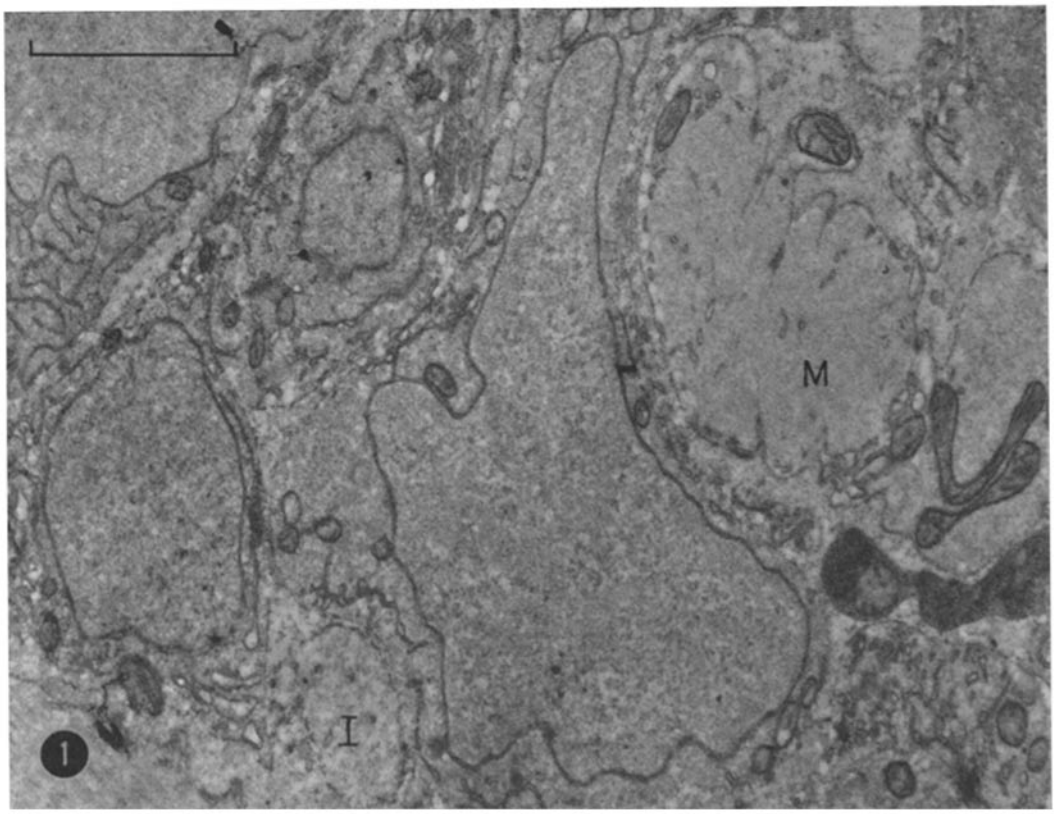
The choice of sodium over potassium permanganate for further investigation of this organism has been made on the basis of slightly, but significantly better preservation of the integrity and continuity of plasma membranes. A comparison

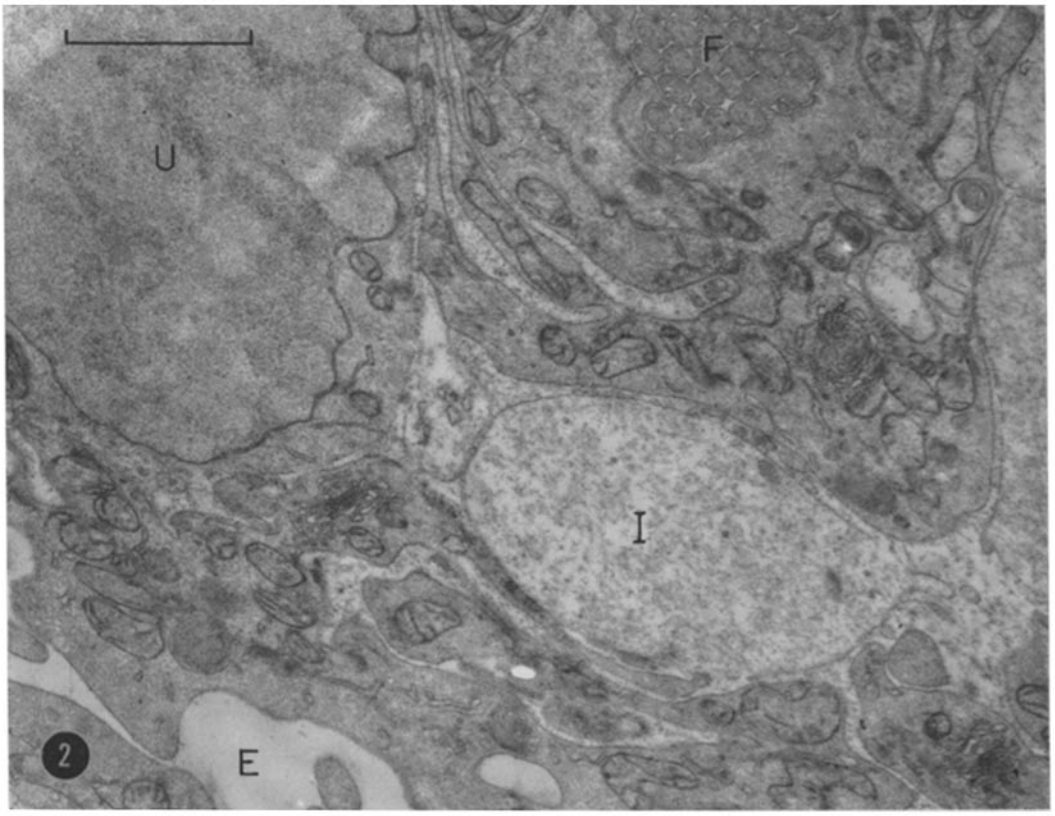
FIGURE 1

This is a general view of planarian parenchyma after potassium permanganate fixation. Illustrated here is a muscle cell (*M*), a transversely sectioned interstitial cell process (*I*), and portions of several unspecialized parenchymal cells. It is indeed curious that a cell's plasma membrane may appear seriously disrupted after potassium permanganate fixation, though the nuclear envelope, mitochondria, and endoplasmic reticulum of that same cell are in a fine state of preservation. × 26,400.

FIGURE 2

Sodium permanganate-fixed planarian parenchyma, illustrating improved plasma membrane preservation. This view includes transversely sectioned cilia of a flame cell (*F*), a portion of longitudinally sectioned excretory duct (*E*), an unspecialized parenchymal cell (*U*), and several interstitial cell processes (*I*). The basal membranes of both the flame cell and the excretory duct cell are extensively convoluted, presenting a greatly expanded surface for exchange between these cells and the surrounding intercellular spaces. Note the poor preservation of ciliary filaments. × 23,100.





of the discontinuous cytoplasmic membranes shown in potassium permanganate–fixed material (Fig. 1) with the relatively intact plasma membranes of sodium permanganate–fixed tissues (Figs. 2 to 4) should illustrate this point satisfactorily. Due to the complexity and lack of orderly arrangement of planarian parenchyma, problems of orientation and interpretation depend heavily on an ability to trace limiting membranes for considerable distances. In this respect, sodium permanganate fixation has proven to be more useful than potassium permanganate fixation.

Three principal disadvantages have been encountered with the use of sodium permanganate. Certain cytoplasmic inclusions which show perfectly good coherence and preservation in osmium tetroxide preparations consistently show shattering and disintegration after fixation with either of the permanganates. This has been the case with rhabdites, and with pigment granules of the eye cup. It should be noted specifically that flame cell ciliary filaments have not been satisfactorily preserved by either sodium or potassium permanganates, though they are clearly demonstrable after osmium tetroxide fixation.

Another somewhat disadvantageous feature is the complete absence of RNP particles in material fixed with either of the permanganates, thus precluding valuable inferences which can often be drawn from the disposition of these particles when they are preserved. The two difficulties mentioned thus far can be surmounted only by the conscientious use of control material fixed with osmium tetroxide.

The third principal disadvantage in the use of sodium permanganate consists in the moderately low contrast observed in Araldite-embedded preparations. Unfortunately, this situation hinders orientation and the determination of focus on the screen of the electron microscope, but the contrast can readily be regained in the course of photographic reproduction, and by the use of "contrast" plates. Staining thin sections of sodium permanganate–fixed material in a solution of potassium permanganate (6) markedly improves the contrast in the specimen; thus any difficulties arising from a lack of contrast in sodium permanganate–fixed material are easily and satisfactorily overcome.

The effects of sodium permanganate fixation on other tissues remain untested at the time of this report. Nevertheless, the results obtained with delicate Turbellarian tissues would seem to recommend this reagent as a possible alternative to conventional fixing agents when the latter yield unsatisfactory results.

SUMMARY

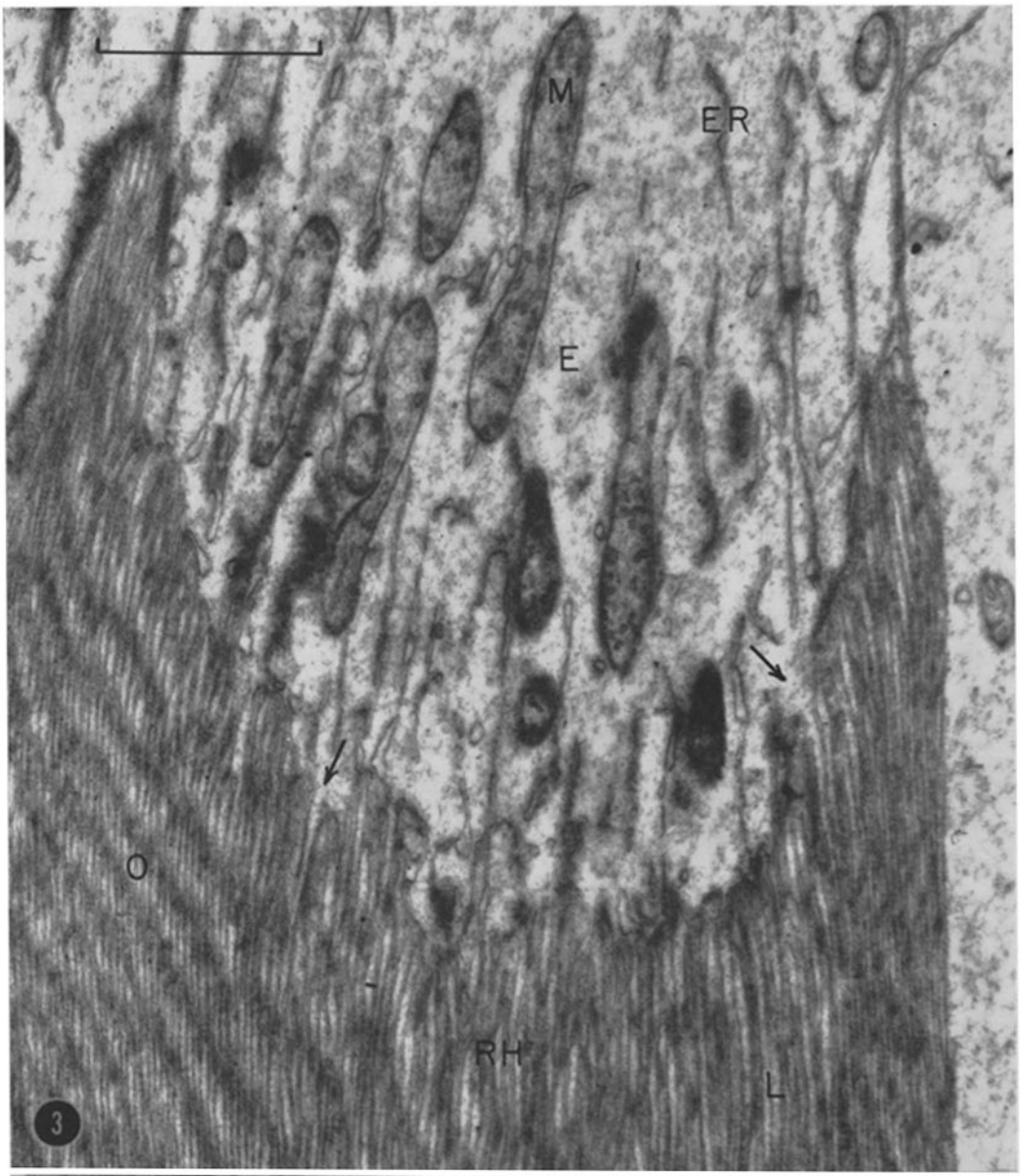
Sodium permanganate has yielded sufficiently improved plasma membrane preservation in tissues of *Dugesia tigrina* to warrant its use as a fixative, despite reduction in specimen contrast, and the disadvantages it shares with potassium permanganate. Improved ion balance in the fixing fluid may explain its superiority to potassium permanganate. The best results obtained with this fixative indicate the use of Araldite embedding procedures and, in conjunction, the staining of thin sections with potassium permanganate.

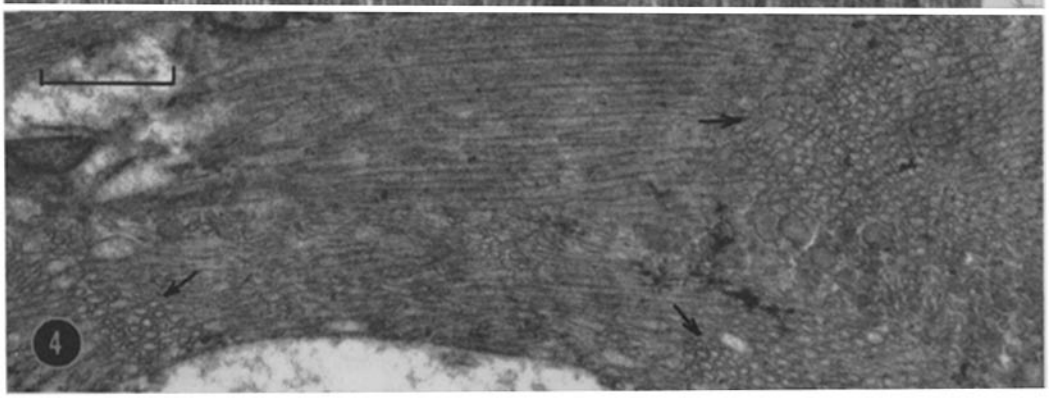
FIGURE 3

Distal portion of a sodium permanganate–fixed planarian retinula cell. The intraocular expansion (*E*) of the cell process contains numerous large mitochondria (*M*), and a considerable amount of endoplasmic reticulum (*ER*). Note that the cytoplasm is continuous (arrows) with the more distal portions of the cell, variously known as the rhabdome, rod border, or rhabdomal lamellae (*RH*). It should be emphasized that this zone does not consist of parallel lamellae as previously reported (11), but is comprised of an array of tubules shown here in longitudinal (*L*) and oblique (*O*) section. There are strong indications that these tubules represent extraordinarily elongate microvilli arising from the expanded portion of the retinula cell process, but conclusive evidence is presently lacking. × 29,300.

FIGURE 4

Section of the rhabdomal portion of a sodium permanganate–fixed planarian retinular cell illustrating (at arrows) circular profiles of transversely sectioned tubules which comprise this portion of the cell. × 17,600.





I wish to express the deepest gratitude to my professor, Dr. George B. Chapman, for his most careful instruction and patient guidance throughout the preparation of this paper.

This investigation was carried out during the tenure of a Predoctoral Fellowship from the National Cancer Institute, United States Public Health Service. Support was also provided by United States Public Health Service Training Grant CRT-5064 to Dr. George B. Chapman.

Received for publication, August 29, 1960.

REFERENCES

1. BRADBURY, S., and MEEK, G. A., A study of potassium permanganate "fixation" for electron microscopy, *Quart. J. Micr. Sc.*, 1960, **101**, 241.
2. DALTON, A. J., and FELIX, M. D., A chrome-osmium fixation for electron microscopy (abstract), *Anat. Rec.*, 1955, **121**, 281.
3. DALTON, A. J., and ZEIGEL, R. F., A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 409.
4. GLAUERT, A. M., and GLAUERT, R. H., Araldite as an embedding medium for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 191.
5. KLUG, H., Uber die funktionell Bedeutung der Feinstrukturen der exokrinen Drusenzellen (Untersuchungen an *Euplanaria.*), *Z. Zellforsch. u. mikr. Anat.*, 1960, **51**, 617.
6. LAWN, A. M., The use of potassium permanganate as an electron-dense stain for sections of tissue embedded in epoxy resin, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 197.
7. LUFT, J. H., Permanganate—a new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
8. MICHAELIS, L., Der Acetat-veronal Puffer, *Biochem. Z.*, 1931, **234**, 139.
9. PALADE, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.
10. PEDERSEN, K. J., Some features of the fine structure and histochemistry of planarian subepidermal gland cells, *Z. Zellforsch. u. mikr. Anat.*, 1959, **50**, 121.
11. PEDERSEN, K. J., Cytological studies on the planarian neoblast, *Z. Zellforsch. u. mikr. Anat.*, 1959, **50**, 799.
12. PRESS, N., Electron microscope study of the distal portion of a planarian retinular cell, *Biol. Bull.*, 1959, **117**, 511.
13. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.
14. WILLIS, J. H., Doctor's thesis, Department of Biology, Harvard University, 1960.