Permanganate Fixation of Plant Cells

By HILTON H. MOLLENHAUER, Ph.D.

(From The University of Texas Southwestern Medical School, Dallas)

PLATES 222 TO 224

(Received for publication, June 22, 1959)

ABSTRACT

In an evaluation of procedures explored to circumvent some of the problems of osmium tetroxide-fixation and methacrylate embedding of plant materials, excised segments of root tips of *Zea mays* were fixed for electron microscopy in potassium permanganate in the following treatment variations: unbuffered and veronal-acetate buffered solutions of 0.6, 2.0, and 5.0 per cent KMnO₄ at pH 5.0, 6.0, 6.7, and 7.5, and temperatures of 2-4°C. and 22°C. After fixation the segments were dehydrated, embedded in epoxy resin, sectioned, and observed or photographed. The cells of the central region of the rootcap are described. The fixation procedures employing unbuffered solutions containing 2.0 to 5.0 per cent KMnO₄ at a temperature of 22°C. gave particularly good preservation of cell structure and all membrane systems. Similar results were obtained using a solution containing 2.0 per cent KMnO₄, buffered with veronal-acetate to pH 6.0, and a fixation time of 2 hours at 22°C. The fixation procedure utilizing veronal-acetate buffered, 0.6 per cent KMnO₄ at 2-4°C. and pH 6.7 also gave relatively good preservation of most cellular constituents. However, preservation of the plasma membrane was not so good, nor was the intensity of staining so great, as that with the group of fixatives containing greater concentrations of KMnO₄. The other fixation procedures did not give satisfactory preservation of fine structure. A comparison is made of cell structures as fixed in KMnO₄ or OsO₄.

INTRODUCTION

In the recent development on electron microscopy, osmium tetroxide has been generally accepted as the most useful fixative and methacrylate has become the usual embedding medium. Osmium tetroxide has long been a standard fixative in animal cytology, but it has never proved so useful in plant cytology. The fact that animal tissues may be embedded in methacrylate with fewer technical difficulties than most plant tissues or organs has also contributed to limiting progress in the electron microscopy of plant materials.

With full recognition of the desirability of adapting osmium tetroxide-fixation successfully to plant materials for electron microscopy, an investigation was made of the suitability of other fixatives. Among these was potassium permanganate which has been used occasionally as a fixative or stain for optical microscope cytology (Schmidt, 1936) and which has been explored as a fixative for electron microscopy by Luft (1956). As used in this laboratory as a fixative for the root tips of *Zea mays*, KMnO₄ gave apparently good results in preliminary investigations. Accordingly, a rather detailed study of variables in KMnO₄ fixation was undertaken.

**Materials and Methods**

The experimental material for this study was the apical 3 mm. of the primary root of *Zea mays*. Seed were germinated on moist filter paper in the dark at room temperature (about 22°C.) and the seedlings allowed to grow under the same conditions for 5 days from "planting." At this time the primary roots were about 9 cm. long.

Longitudinal sections approximately 0.2 mm. thick were excised from known positions along the axis of the apical 3 mm. of the root tips and placed immediately into one of the fixatives indicated in Table I.

The sections were transferred from the fixative to 25 per cent ethanol and then, at hourly intervals, to 50, 70, and 90 per cent ethanol and two changes of absolute ethanol. The temperature of the 25 per cent and the 50 per cent ethanol was kept at the temperature of the
PERMANGANATE FIXATION OF PLANT CELLS

TABLE I

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>PH</th>
<th>0.50 per cent KMnO₄ for 2 hrs</th>
<th>2.00 per cent KMnO₄ for 2 hrs</th>
<th>5.00 per cent KMnO₄ for 2 min</th>
<th>5.00 per cent KMnO₄ for 75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4</td>
<td>XX</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>XX</td>
</tr>
<tr>
<td>5.0</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>X</td>
<td>X</td>
<td>XX</td>
<td></td>
<td>XX</td>
</tr>
<tr>
<td>6.7</td>
<td>XX</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Unbuffered</td>
<td>X</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
</tbody>
</table>

Note: Only the fixation procedures which appeared to give especially good preservation of cell fine structure are discussed in this paper. These are marked xx. The fixatives for which specific pH is indicated were buffered with veronal-acetate buffer (see Palade, 1952). The unbuffered fixatives contained only distilled water and KMnO₄. Some fixatives containing 0.05 per cent KMnO₄ and 0.10 per cent KMnO₄ were also used in this investigation. However, because of the extremely poor preservation of cell fine structure obtained in preliminary tests, this phase of the investigation was not pursued in detail. The effects of using buffered 5.0 per cent KMnO₄ were also investigated to a limited extent. The results of these preliminary tests were generally similar to those described for the corresponding treatment in the 2.0 per cent KMnO₄. Root tips from three or more “plantings” of maize were separately fixed and embedded for each of the fixation procedures listed in the above table. Thin sections from at least three root tips from each “planting” were used in obtaining the results reported herein.

Four types of cells were originally considered in this study: (1) the outermost cells of the rootcap; (2) the central cells of the rootcap; (3) the most apical cells of the root proper, the promeristematic cells; and (4) a succession of epidermal cells. The fine structure of these cell types is discussed in other papers from this laboratory (Whaley et al., 1959). However, only the central region cap cells are considered here. Fixation of this cell type is generally representative of that of most cells in the root tip. This cell type is described as it is seen in micrographs of sections fixed in unbuffered 5.0 per cent KMnO₄ for 2 minutes at 22°C. For simplicity of expression, this fixation is referred to as the 5.0 per cent KMnO₄ fixation.
to throughout this paper as the standard fixation. The fine structure seen with use of this fixation is then compared with that of cells of the same type after treatment by the four other fixation procedures that produced relatively good results ("xx" in preceding tabular summary of protocol).

5.0 Per Cent KMnO₄, Unbuffered, 2 Minutes at 22°C.—(standard fixation). The ground substance of the cytoplasm appears to be composed of relatively fine granules and seems homogeneous (Figs. 1 and 2). There is good definition of an endoplasmic reticulum (er in the figures), mitochondria (m), distinct Golgi apparatus (ga), and bodies of the same general order of magnitude as the mitochondria, but with different internal structure (pp). No organized structural detail can be seen in the nuclei (n). The nuclear envelope appears as a double-membrane structure with numerous distinct pores (np) (Fig. 1). The endoplasmic reticulum is apparently continuous by contact, if not by actual extension, from one cell to the next through the plasmodesma (p). This treatment does not reveal the details of wall structure (w). However, continuities across the walls are clearly visible and the thicker longitudinal walls show sufficient differences in density to indicate that structural differentiation exists in the wall (Figs. 2 and 4). At the magnifications used here (× 11,000 to 18,000), the plasma membrane appears to be a single layer with occasional irregularities which may include infoldings that course around granules or other small bodies (Fig. 2). In most cells from the central region of the rootcap, elements of the endoplasmic reticulum appear to run parallel to the plasma membrane (Figs. 1, 2, and 4). In the cytoplasm there are numerous irregularly shaped masses of electron-dense substance (i) (Figs. 1 and 2). In some micrographs these masses appear to be bounded by a single membrane (Fig. 2). Two distinct types of unidentified cytoplasmic bodies are seen in these cells. One, (i), is a spheroidal body approximately 0.5 μ in diameter, which, at the magnifications used, appears to be bounded by single membrane (Fig. 2). This body has no apparent organized internal structure but it contains a finely granular substance. The other unidentified body, (ii), is of the same order of magnitude and also appears to be bounded by a single membrane (Figs. 1 and 2), but it shows no internal structure or contents.

The bodies noted above in the size range of the mitochondria have bounding membranes that seem more electron-dense and are probably thinner and spaced more closely together than those of the mitochondria. The absence of an extensive internal organization contrasts sharply with the mitochondria (Figs. 1 and 2). In agreement with Setterfield et al. (1959) and others, these structures have arbitrarily been designated as proplastids.

The central rootcap region cells contain what are here presumed to be starch-storing bodies to which the name amyloplast (a) is applied as it has been by Setterfield et al. (1959). Two or three starch grains (st) are sometimes apparent in these amyloplasts, which appear to be bounded by a double membrane of the same order of magnitude as that of the proplastids (Fig. 4).

5.0 Per Cent KMnO₄, Unbuffered, 75 Minutes at 22°C.—Cells treated with this fixation procedure are morphologically similar in appearance to those treated with the standard fixation but differ from them in the increased electron opacity of certain cell constituents. With this treatment the starch grains of the amyloplasts appear particularly dense (Fig. 3). The limiting membrane of the cytoplasmic body designated ii (Fig. 3 a) is thicker than that with the previously described fixation. Some differentiation of chromatin from the ground substance of the nucleus is apparent in many of the nuclei and the Golgi vesicles (gv) often appear with an electron-dense internal region (Fig. 3). The cell walls have a higher electron opacity and some appear striated, thus indicating structural differentiation of the kind seen in micrographs of shadowed material (Frey-Wyssling, 1953; Bayley et al., 1957).

2.0 Per Cent KMnO₄, Unbuffered, 2 Hours at 22°C.—Cells fixed by this procedure are similar in structural detail to the same cell types treated with the standard fixative. As with the preceding fixative, some of the cell constituents exhibit greater electron opacity than that with the standard fixative. The electron opacity is, however, not quite so great as that with the preceding fixative (Fig. 6). With this fixation procedure there is considerable distinction between the membranes of the mitochondria and those of the proplastids with respect to electron density (Fig. 6).

2.0 Per Cent KMnO₄, Veronal-Acetate Buffer, pH 6.0, 2 Hours at 22°C.—Cells fixed with this buffered solution reveal no significant differences in structure from the same cell types fixed by the standard procedure. The electron density of the
cell components is similar to that in cells fixed for 2 hours in unbuffered 2.0 per cent KMnO₄.

- **0.6 Per Cent KMnO₄, Veronal-Acetate Buffer, pH 6.7, 2 Hours at 2–4°C.**—Cells fixed with this buffered solution do not show the irregularly shaped masses described for the standard fixation. Instead, nearly spherical, vacuole-like structures (v) which contain small amounts of distributed, granular substance are present (Fig. 5). These vacuole-like structures are apparently bounded by a single membrane and their distribution in the cell is similar to that of the irregularly shaped masses. With this fixation procedure the plasma membrane is not clearly defined, though it is perceptible in many micrographs (Fig. 5). There is good definition of the other cytoplasmic inclusions such as mitochondria, Golgi apparatus, endoplasmic reticulum, proplastids, the unidentified cytoplasmic bodies (i) and (ii), and the nuclear envelope. No organized structural detail can be seen in the nuclei. The ground substance of the cytoplasm is perhaps somewhat more homogeneous than that described for the standard fixation.

**DISCUSSION**

Micrographs of KMnO₄-fixed plant cells contrast sharply in some features with micrographs of OsO₄-fixed plant cells. Nonetheless the basic structure is quite comparable in cells prepared by the two fixatives. This fact, together with the observation that some structures are more clearly demonstrated by permanganate fixation, indicates an important usefulness for this fixative for both plant and animal cells. Shrinkage in permanganate-fixed cells does not appear to be greater than, if indeed it is as great as, in osmium tetroxide-fixed cells. The structure of the cell wall is not satisfactorily demonstrated in micrographs of materials prepared with either fixative.

In osmium tetroxide-fixed plant cells the ground substance of the cytoplasm appears very complex, and contains numerous small particles and granules which seem variously agglomerated (Chayen and Jackson, 1957; Setterfield et al., 1959). In permanganate preparations the ground substance has a fine granular appearance and seems homogeneous. Some of the granules seen in osmium tetroxide-fixed materials apparently are not demonstrated in permanganate-fixed materials. The absence of these granules contributes to the manner in which the major cytoplasmic components stand out in permanganate preparations.

One of the principal differences between cells fixed in osmium tetroxide and in permanganate is in the appearance of the endoplasmic reticulum. As noted by Luft (1956), permanganate seems specific for cell membranes. In permanganate-fixed plant cells the endoplasmic reticulum is well defined and its continuity throughout the cytoplasm and from cell to cell is clear. In osmium tetroxide-fixed material, segments of an endoplasmic reticulum have been demonstrated by numerous investigators (Wyckoff, 1954; Porter, 1957; Lund et al., 1958; Setterfield et al., 1959) but the continuity of the structure is not apparent. With permanganate fixation the profiles of the endoplasmic reticulum always appear smooth and the “Palade granules” are not seen. The failure of permanganate to demonstrate these granules was noted by Luft (1956).

The nuclear envelope is readily seen in osmium tetroxide preparations but its double-membrane character, the numerous pores, and its continuity with the endoplasmic reticulum are much clearer in permanganate preparations. The cisternae and vesicles of the Golgi apparatus appear more clearly in permanganate-fixed cells although the basic structure is the same with either fixative (Whaley et al., 1959; Heitz, 1957; Perner, 1957; Buvat, 1958). The mitochondria appear similar with both fixatives but again, better definition of the membranes by permanganate fixation makes them stand out more distinctly. In a like manner the proplastids, and other membrane-bound inclusions, are more distinct in permanganate preparations.

The plasma membrane is sensitive to variations in the permanganate fixation procedure but is somewhat better demonstrated in permanganate preparations than in osmium tetroxide preparations. The irregularly shaped masses (l in Figs. 1 and 2), tentively unidentified in character, are readily demonstrated in permanganate preparations. The appearance of these masses is dependent on the concentration of the KMnO₄.

In the central rootcap cells, the nucleolus is not clearly differentiated from the rest of the nuclear material in permanganate preparations, but stands out clearly in osmium tetroxide preparations. Chromatin is well defined in permanganate.
preparations only with long fixation and relatively high concentrations of KMnO₄ (for example, 2.0 per cent KMnO₄ for 2 hours). The picture of chromatin obtained also depends on pH but the relationship of pH to the other factors has not been sufficiently explored.

The amyloplasts of rootcap cells show the same basic pattern with both fixatives. The dense granules of the amyloplasts reported by Setterfield et al. (1959) in osmium tetroxide-fixed wheat embryo cells are not apparent in these permanganate-fixed cells. When cells are fixed in high concentrations of KMnO₄ for long periods at room temperature, the starch grains in the amyloplasts "stain" darkly. At lower concentrations, shorter times, lower temperatures, or a combination of these, the starch grains show up less dense to the electron beam and the permanganate-fixed material then closely resembles osmium tetroxide-fixed material. Under any of these fixation conditions the membranes of the permanganate-fixed amyloplasts are more distinct than those of osmium tetroxide-fixed amyloplasts.

The results of the use of these permanganate fixation procedures with animal tissues will be reported elsewhere. The general use of permanganate to fix animal cells has the same advantages and disadvantages as for plant cells.

BIBLIOGRAPHY

Perner, E. S., Naturwissenschaften, 1957, 44, 336.
EXPLANATION OF PLATES

All figures are electron micrographs of longitudinal sections of the root tip of Zea mays and show cells of the central rootcap region. The lettering code used to identify the cell constituents is:

- a: amyloplast
- ch: chromatin
- er: endoplasmic reticulum
- go: Golgi apparatus
- gv: Golgi vesicles
- i: unidentified cytoplasmic body
- ii: unidentified cytoplasmic body
- l: irregularly shaped mass
- m: mitochondrion
- n: nucleus
- np: nuclear pore
- p: plasmodesma
- pp: proplastid
- s: starch grain
- st: starch grain
- v: vacuole-like structure
- w: cell wall

PLATE 222

Fig. 1. Portions of two cells of the promeristematic region are seen in the upper part of the micrograph and portions of two cells of the central region of the rootcap in the lower part of the micrograph. The cell wall marked w separates the root proper from the rootcap. Fixation in unbuffered 5.0 per cent KMnO₄ for 2 minutes at 22°C. X 17,000.
(Mollenhauer: Permanganate fixation of plant cells)
PLATE 223

Fig. 2. Portions of cells of the central rootcap region approximately 3 μ from the most apical root cells. Fixation in unbuffered 5.0 per cent KMnO₄, for 2 minutes at 22°C. X 11,000.

Fig. 3. Portion of a rootcap cell containing an amyloplast with three starch grains of high electron density. The Golgi vesicles and the cell wall also show the increased density characteristic of this fixation procedure. Fixation in unbuffered 5.0 per cent KMnO₄, for 75 minutes at 22°C. X 18,000.

Fig. 3a. Portion of a rootcap cell containing two unidentified cytoplasmic bodies ii. The wall thickening and the density differences in the wall are characteristic of this fixation procedure. Fixation is the same as in Fig. 3. X 17,000.

Fig. 4. Portions of three rootcap cells, one containing three amyloplasts with starch grains of low electron density. Fixation in unbuffered 5.0 per cent KMnO₄, for 2 minutes at 22°C. X 17,000.
Mollenhauer: Permanganate fixation of plant cells
Plate 224

Fig. 5. Cells of the promeristematic region at the upper right of the micrograph and cells of the central region of the rootcap at the left, separated by the cell wall marked w. The irregularly shaped masses (l) shown in Figs. 1 and 2 are not demonstrated by this fixation procedure. There are, however, numerous vacuole-like structures which appear to correspond to the irregularly shaped masses and to be characteristic of this fixation procedure. Fixation in 0.6 per cent KMnO₄, buffered with veronal-acetate to pH 6.7, 2 hours at 2-4°C. × 15,000.

Fig. 6. Cells of the central region of the rootcap, showing at the right a difference in density between the mitochondrial membranes and those of the proplastids and at the left chromatin distinguished from the ground substance of the nucleus. Fixation in unbuffered 2.0 per cent KMnO₄ for 2 hours at 22°C. × 16,000.
(Mollenhauer: Permanganate fixation of plant cells)