Effects of Varying the Vehicle for OsO_4 in Tissue Fixation. BY JAMES B. CAULFIELD.* (*From The Rockefeller Institute for Medical Research*).‡

It is well known that the quality of OsO_4 fixation of tissue cells obtained with any one of the several recommended mixtures (1-3) may vary considerably in the same block or from one tissue to another. In some cells of a sample, the material of the cytoplasmic matrix may appear as an even distribution of particles and finely fibrous elements, in which case it is regarded as well fixed. In other cells in the same sample the matrix components may be condensed into clumps of variable size and density, separated by areas of lesser density devoid of any resolvable structure. Such clumping is usually taken as descriptive of relatively poor fixation, since little or no evidence of it can be detected in equivalent living cells (4). It has been noted also that in images of what one considers to be well fixed cells, the contours of the membrane-limited elements of the endoplasmic reticulum are smooth, whereas in another part of the same preparation equivalent structures may appear angular or irregular and vesiculated. In some images the nucleoplasm is homogeneous and relatively uniform in density, whereas in others it is obviously uneven and coagulated. Some of these differences may be referable to structural or compositional variations associated with functional changes, but in most instances the differences seem more properly considered as products of fixation. Especially is this true in frequent instances where the differences noted are found at different levels in the same block, and are easily related to unequal conditions of fixation at different depths in the block.

It has become apparent that the tonicity of a fixative has a direct effect on the appearance of the fixed tissues (2, 5). In general, cells fixed in solutions of low tonicity show swollen mitochondria, etc., while the reverse is true where the tonicity is high. Some effort has been made to put this on a rational basis by Rhodin and Zetterqvist (2, 5), but direct measurements of intact cells indicate that their tonicity is in some cases considerably different than that of plasma. Values reported for plasma range from 0.31 to 0.34 osmolar (6–8). Opie (9) reported kidney cortex cells to be isotonic with 0.23 M NaCl and liver parenchymatous cells isotonic with 0.34 M NaCl. We note that the osmolarity of NaCl in such solutions is 0.43 and 0.63 respectively.

Since so little is known about intracellular osmolarities and the physiological variations these may undergo and because of the great difficulty in calculating osmolarities, particularly in a complex buffer system near neutrality (10), any choice of a molar concentration for a fixative is bound to be arbitrary. However, given an arbitrary concentration of solutes, consistent variations in either direction from this figure may be obtained and images of tissue fixed in the various solutions may be compared. Thus the quality of fixation becomes the important criterion for determining the composition of any fixative used.

In a recent study of dividing tumor cells the available fixatives were found

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to provide less than adequate preservation of fine structure in all parts of the tissue block except in a narrow zone a few cell layers in from the surface. Since the number of cells in mitosis is never large, to have the number available for study further restricted by poor fixation considerably hampered the progress of the investigation. An attempt was therefore made to explore the properties of a few reasonable variants of the standard mixtures.

Increasing the tonicity of the OsO_4 vehicle (veronal acetate as described by Palade) by the addition of NaCl as suggested by Rhodin (2) resulted in certain fairly well defined and constant changes in the appearance of a variety of cells. Certain aspects of morphology appeared to be more truly maintained, whereas some of the effects of the NaCl resulted in definite deterioration of structure.

A natural step from the foregoing is to increase the tonicity of the OsO_4 vehicle to an arbitrary point with a nonionic substance which is able to pass freely into and out of the cell and nucleus. Sucrose was the substance of choice, although previously it has been tried with some benefit in certain type cells (11). Only the results obtained by adding sucrose in varying concentrations to the basic fixative of Palade (1) will be reported, since it provided the best general fixation of any method tried.

Animal tissues were fixed for 1 hour at 0°–4°C. (2) in a solution of 5 ml. of stock buffer (14.714 gm. Na veronal plus 9.714 gm. Na acetate · $3H_2O$, diluted to 500 cc.), 5 ml. 0.1N HCl, 2.5 ml. H_2O, and 12.5 ml. 2 per cent OsO_4. The pH was adjusted to 7.4 when necessary by the addition of a few drops of the 0.1N HCl or stock buffer. To this was added 0.045 gm. of sucrose per ml. of fixative.

Plant tissues were fixed 30 minutes at 0–4°C. in a solution containing 1 ml. of the stock buffer solution, 1 ml. of 0.1N HCl, 2 ml. of H_2O, and 4 ml. of 2 per cent OsO_4. The pH was adjusted to 7.4 when necessary. 0.015 gm. of sucrose per ml. of fixative was then added. In these experiments the dehydration and embedding were kept constant (1). A positive effort was made to utilize sections showing similar interference colors. All sections were cut with glass knives or a diamond knife of Fernández-Morán (12) mounted in a Porter-Blum microtome (13, 14).

The tissues examined include pancreas, muscle, kidney, central and peripheral nervous system, retina, skin, ovary, testis, bone marrow, solid and ascitic tumor cells, and two plant tissues (onion and bean root tips).

The basic appearance of tissue cells fixed with sucrose as an additive is similar to the appearance of tissue fixed in a variety of OsO_4 vehicles. The intercellular cohesion is well preserved, there being few retraction spaces. Rough surfaced elements of the endoplasmic reticulum are fairly evenly disposed with few sharp angular profiles and the spaces between the opposing membranes are regular. Profiles of the smooth surfaced endoplasmic reticulum are free of angulations. The limiting membranes of the mitochondria are somewhat smoother than with previous fixatives. Mitochondrial cristae and matrix appear as in other procedures. Between the formed elements the cytoplasmic matrix of fine fibrillar and granular material shows much less coagulation than in previous methods of tissue preparation. Aggregation of nuclear constitutents is less pronounced in the presence of sucrose. This results in a more even distribution of electron-scattering material and much fewer areas that appear empty and devoid of dense material (Figs. 1 to 3).

The addition of sucrose to the fixing vehicle, besides providing a general improvement in the quality of fixation, increased the amount of well fixed tissue in each block. Usually the outer two or three cell layers of a tissue block are quite distorted. This alteration appears to be due to the dehydration and embedding techniques employed and is essentially unchanged with a wide variety of fixation procedures. With sucrose as an additive to the OsO_4 solution, all depths of the block are quite well fixed provided the block is not more than 2 mm. on any side.

In plant cell fixation there is little or no separation of the cytoplasm from the cellulose wall. The nuclear envelope and contents are similar to animal cells. The criteria of good fixation, *i.e.* evenly spaced membranes, lack of clumping, etc. when applied to the plant cell images would indicate that adequate fixation has been attained.

Tissues vary in many respects and no single fixative can be reasonably expected to give good results with all. Of the methods and tissues tried the sucrose-containing ones provided better fixation, except for central and peripheral nervous tissues and kidney tubular cells.

CONCLUSIONS

1. Sucrose, when added to the standard Palade fixative to increase tonicity without increasing Na ions, frequently results in better preservation of larger portions of the block than the other fixation methods evaluated.
2. The method has, moreover, been found to be adequate for fixing onion and bean root tips.

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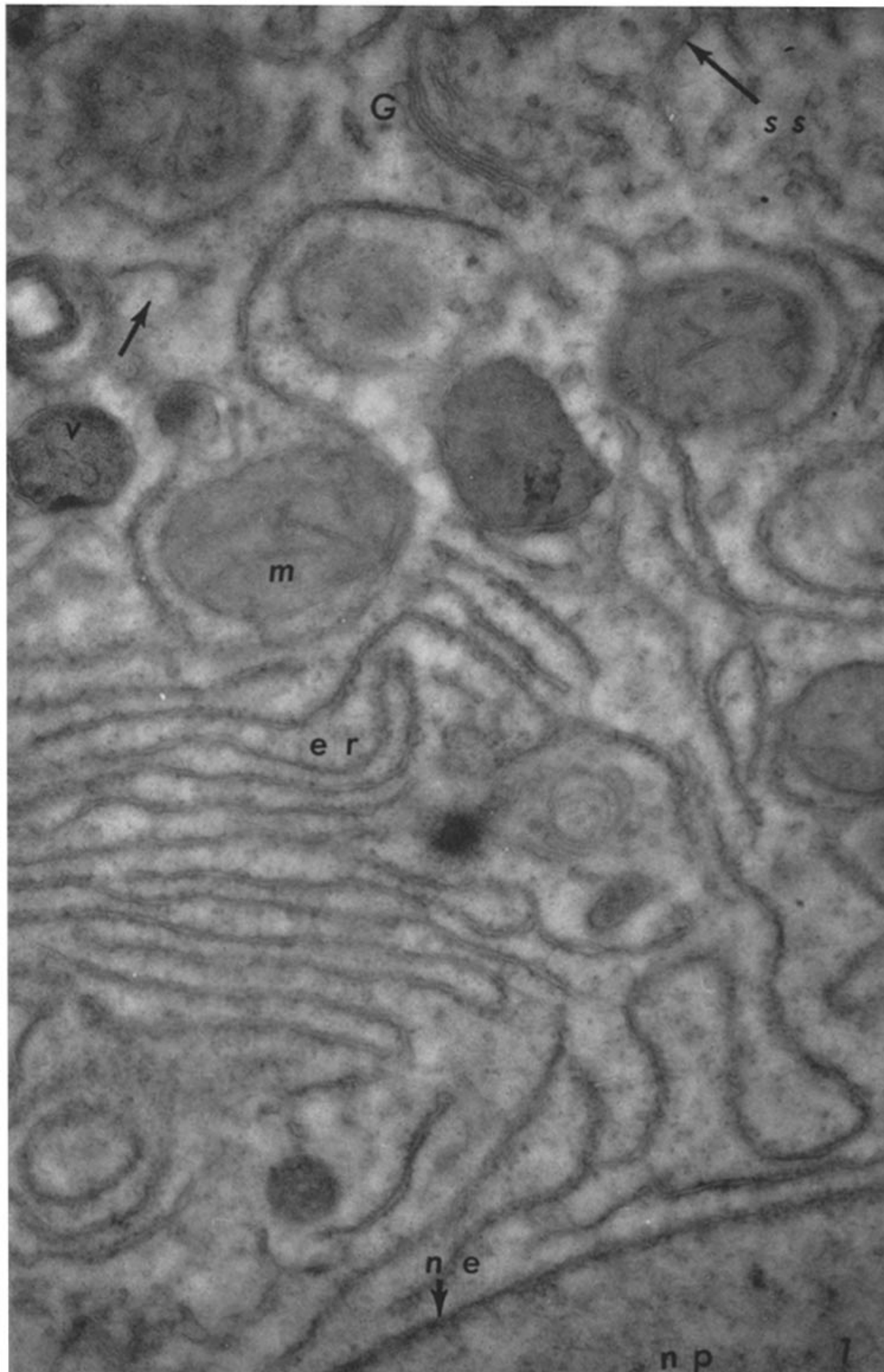
Explanation of Plates

Plate 260

Fig. 1. Micrograph of normal rat liver fixed in buffered OsO_4 to which sucrose had been added. The nucleoplasm (*np*) is homogeneous and evenly distributed. There are no dense aggregates of material nor any areas showing diminished electron scattering. The plane of section is such that neither the nuclear pores nor the doubleness of the nuclear envelope (*ne*) is evident. The cytoplasm abuts the nuclear membrane evenly. There is no evidence of shrinkage spaces or areas in which the nuclear envelope bulges. The endoplasmic reticulum (*er*) is disposed in undulations free of angulations. The distance between members of membrane pairs is fairly uniform. Smooth surface (*ss*) forms of the endoplasmic reticulum are represented as well as a close lamellar system, the Golgi component (*G*). The mitochondria (*m*) have smooth contours. The cristae and the limiting membranes are evenly disposed and characteristic of liver. The mitochondrial matrix is granular and homogeneous. At *v* a body (hemosiderin granule) containing an aggregate of fine dense particles representing ferritin can be seen. Throughout the cytoplasm similar small dense particles are in evidence (arrow). × 39,000.

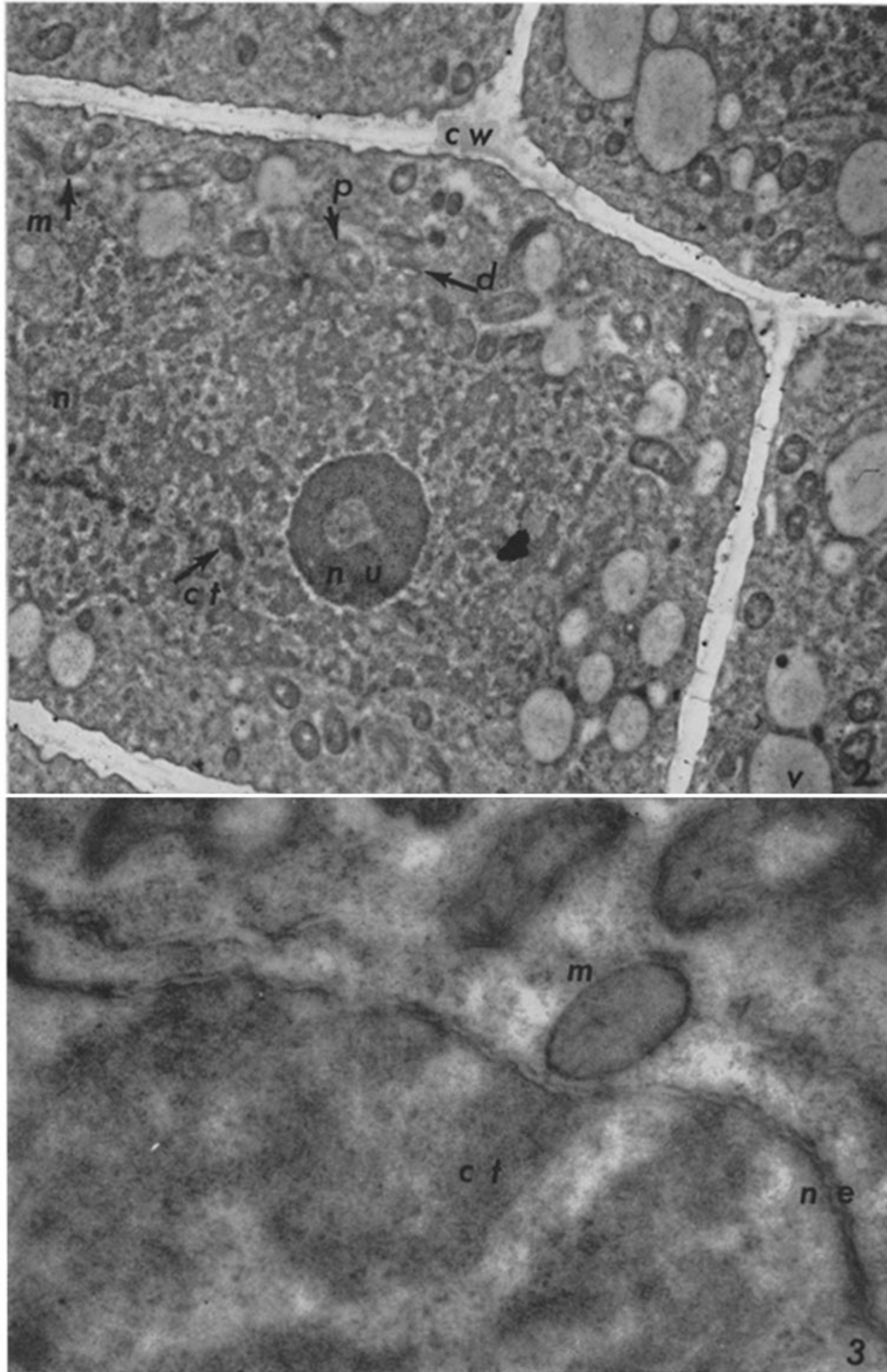


(Caulfield: OsO_4 in tissue fixation)

PLATE 261

FIG. 2. This low power picture of onion root tip shows the general distribution and cellular contents in a region slightly above the actual growing cap, but well within the region of high mitotic activity. The cellulose wall (*cw*) is visible, but its two major components are not clearly defined in this picture. The cytoplasmic organelles, mitochondria (*m*), dictyosomes (*d*), vacuoles (*v*), and plastids (*p*) are readily apparent at this low magnification. The vacuoles (*v*) are quite small. As the cell develops, the vacuoles enlarge and have a finely fibrous content of very low concentration. The nucleus (*n*) contains a network of chromatin threads (*ct*) separated by an homogeneous granular appearing material. The nucleolus (*nu*) rather characteristically contains fine dense partcles. These particles are not present elsewhere in either the nucleus or cytoplasm. × 8,000.

FIG. 3. This picture is representative of cells from the undifferentiated zone of the meristem. The chromatin material (*ct*) is seen to be more dense than the interchromatin matrix, but in neither is there any evidence of recognizably formed elements other than randomly placed fibrous and granular material. The nuclear envelope (*ne*) is clearly represented by two dense lines separated by a pale area of constant width. The mitochondria (*m*) have a smooth external limiting membrane. The cristae are distributed randomly. The endoplasmic reticulum is smooth in contour and fairly uniform in dimensions. The granular cytoplasmic matrix is evenly distributed. × 35,500.



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