Brief Notes

Some Experiments with Chromium Compounds as Fixers for Electron Microscopy.* BY FRANK N. LOW AND JAMES A. FREEMAN.‡ (*From the Department of Anatomy, Louisiana State University School of Medicine, New Orleans.*) §

In the course of experimentation with various tissue fixations other than OsO_4 it was noticed that mixtures containing chromium compounds showed considerable promise. Therefore the fixation characteristics of three such compounds, $CrCl_3$, $K_2Cr_2O_7$ and CrO_3 (the anhydride of H_2CrO_4), were systematically investigated. Although the action of all three was essentially similar, CrO_3 proved to be the most satisfactory and was used exclusively in subsequent experiments. Its concentration was varied from 1 to 6 per cent. It was tried in combination with formaldehyde, the concentration of which was varied from 2 to 32 per cent. Sodium chloride, in concentrations never exceeding 3.4 per cent, was used to control tonicity in many experiments. A number of standard buffers were mixed with the solutions without noticeable profit.

The fluid finally adopted as the most satisfactory was an aqueous solution containing 3 per cent chromic oxide (CrO_3), 4 per cent formaldehyde (HCHO) and 0.85 per cent sodium chloride (NaCl). The pH of this mixture was about 3.2. The fixation time was varied from ¼ to 17 hours, but after several hours fixation the tissue resembled a metal-shadowed preparation, (apparently due to gradual deposition of chromium) and so it was decided that a short fixation of ¼ to ½ hour was best. Washing for ½ hour in distilled water immediately after fixation produced "cleaner" preparations. Since cells rich in endoplasmic reticulum such as those of the exocrine pancreas responded most favorably to this type of fixation, these were adopted as test objects in developing the procedures. Occasionally the somewhat less basophilic parotid cells were also used.

After methacrylate embedding the tissue was light yellow or brown with a darker center and the dark areas deep in the tissue usually represented areas of better fixation. The tissue was brittle and hard, but with care could be sectioned in silver ribbons with a Porter-Blum microtome. Despite the common presence of fine chatter good sections were obtained from almost every block.

The general appearance of pancreatic acinar cells fixed by this method is illustrated in Figs. 1 to 4. The spaces within the endoplasmic reticulum appear less dense and alternate throughout most of the cytoplasm with darker bands representing the cytoplasmic matrix. The apparently linear organization of the cytoplasm is characteristic of these cells and has been repeatedly demonstrated with OsO_4 fixation (1, Fig. 9). Zymogen granules are structureless and light (Figs.

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2 and 3) in contrast to their dense appearance after OsO_4 fixation (2, Fig. 8). In many preparations the granules themselves appear to have been extracted, with only their external contours remaining (Fig. 2). Mitochondrial contours are also well preserved (Fig. 3), although their substance is of uniform density and shows no indication of either external membrane or any internal structure as demonstrated by OsO_4 fixation (3, Figs. 4 and 9).

Considerable internal structure is evident in nuclei (Figs. 1, 2). There are irregular light and dark areas, the latter coinciding in distribution with the chromatin of the light microscope image. The nucleoplasm does not show the granular appearance characteristic of OsO_4 preparations (1, Fig. 10). The nucleolus, when present (Fig. 2), may be distinguished by its own characteristic medium density. It is not granular. The region normally occupied by the nuclear membrane is of low density and shows no ultrastructure (Fig. 2), such as the double membrane characteristic of OsO_4 fixation (4, Fig. 8). The outline of the nucleus is usually irregular and since, in both light microscopy and electron microscopy of OsO_4-fixed preparations (2, Fig. 8), the nuclei of exocrine pancreatic cells are uniformly well rounded, this is probably due to shrinkage. Nevertheless other intracellular relationships appear to be well preserved.

The sections in Figs. 1 to 3 are quite thick and the alternating layers of cytoplasmic matrix and endoplasmic reticulum appear as dark and light lines respectively. As greater thinness is achieved (Fig. 4), the dense matrix may be seen to be heavily populated by the small components described by Palade (5). These particles seem chiefly responsible for the density of this part of the cytoplasm. In these preparations their diameters average about 130 A, which corresponds well with Palade's original measurements of 100 to 150 A (5). The membrane known to limit the endoplasmic reticulum (1) is seldom visualized in these chromium preparations and whether or not it is preserved by the present method is doubtful.

As indicated above this fixation technique is not so successful with other types of tissue. Among the representative tissues tried, liver, parotid, and striated muscle fix only moderately well. Good liver cell fixation is obtainable but is only occasionally encountered in a typical block. Areas of cytoplasm containing no organoids or endoplasmic reticulum tend to disintegrate. When this does not occur the cytoplasmic mass is largely structureless and of only slightly varying density. In striated muscle the various bands are discernible as are also the myofilaments. The latter are seen in the A disc and are responsible for most of its density. They cannot be observed to extend into the relatively light I disc. The Z band is dense. In most preparations the myofibrils separate laterally and break up, resulting in a poor appearance. In general the results with muscle have not been good. With other body tissues the results were even less encouraging. Most epithelial and endothelial cells fix poorly. In general it may be said that the less basophilic the cytoplasm, the poorer the fixation. The poorest results are obtained with the most highly hydrated of cells. Frequently, even in pancreas and parotid preparations, there may be groups of adjacent cells, some well fixed, some poorly fixed, and some badly broken up. This reflects a tendency for nearby cells to react individually to the fixer. Erythrocytes and the mucous of goblet cells fix

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very well. Extracellular components of the tissues do not seem to fix at all.

The specific action of the three constituents of this fixer may be analyzed as follows. The essential feature of the fixation is the coagulating action of the CrO_3, which seems to prevent disintegration. This proves to be most effective in the presence of strong cytoplasmic basophilia. The part played by the formaldehyde is most evident in the nuclear fixation, which is never good if the formaldehyde is omitted. However, formaldehyde alone is not an adequate nuclear fixer. The influence of the NaCl is clearest in the cytoplasm. Here the proper amount seems to prevent swelling or shrinkage of the endoplasmic reticulum. The final effects should not be interpreted as resulting from the action of any one of the compounds alone, because the successful action of one somehow depends on the presence of other compounds.

The general picture resulting from fixation with the chromic oxide-formaldehyde-salt mixture corresponds well with that obtained with OsO_4 in some respects and differs markedly in others. The most fundamental difference is the absence of certain membranes in our preparations. These include the nuclear and mitochondrial membranes, those of the cristae mitochondriales, and, possibly also, the membranes of the endoplasmic reticulum. Since these membranes might have been preserved although not visualized, some tissues were osmicated after chromium fixation. The membranes could not be demonstrated although the tissue darkened on osmication in the usual manner. It seems reasonable to conclude that lipoprotein membranes are not preserved by this technique.

Certain results of this new fixation can be useful along analytical lines. For example, the Palade granules were originally demonstrated after both OsO_4 and formaldehyde fixation (5). When they were found to be well shown with the present technique, it was thought possible that the formaldehyde might have been responsible for their preservation. To check this some material fixed in CrO_3 alone was examined. In spite of bad overall fixation the Palade granules were clearly evident. There is, therefore, less reason to doubt their existence as true structural units of the living cell.

While the fixative described in this paper cannot be recommended as generally valuable for electron microscopy, its usefulness as a supplement to OsO_4 mixtures has been indicated in the foregoing paragraphs. It is proposed that concurrent fixation in buffered OsO_4 and chromic oxide-formaldehyde-salt mixture would reveal points of comparison useful in interpreting the ultrastructure of protoplasm.

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Explanation of Plate 161

All tissues illustrated were fixed for ½ hour in an aqueous solution containing 3 per cent CrO_3, 4 per cent formaldehyde, and 0.85 per cent NaCl, followed by washing for ½ hour in distilled water. All are from pancreatic acinar cells of the rat.

Fig. 1. The light and dark areas in the nucleus (*N*) are characteristic. In the cytoplasm the light "cavities" of the endoplasmic reticulum alternate with the dark layers of cytoplasmic matrix. × 14,000.

Fig. 2. The light and dark areas of the nucleus are distinguishable from the nucleolus (*n*) which is of medium density. The nuclear membrane appears as a light line which is otherwise structureless. Zymogen granules (*Z*) are apparently completely dissolved out. The clear intercellular areas suggest some shrinkage. × 14,000.

Fig. 3. Zymogen granules (*Z*) are light and structureless but do not seem to be so completely dissolved out as in Fig. 2. Mitochondrial contours (*M*) seem well preserved but no external membrane or internal structure is visible. × 18,000.

Fig. 4. The light "cavities" of the endoplasmic reticulum alternate with the darker layers of cytoplasmic matrix. Palade granules are visible in the latter. A portion of a zymogen granule (*Z*) is shown at the right. × 110,000.

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PLATE 161
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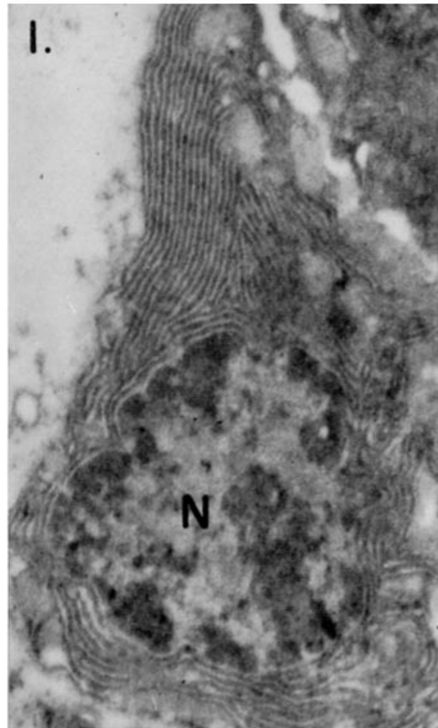

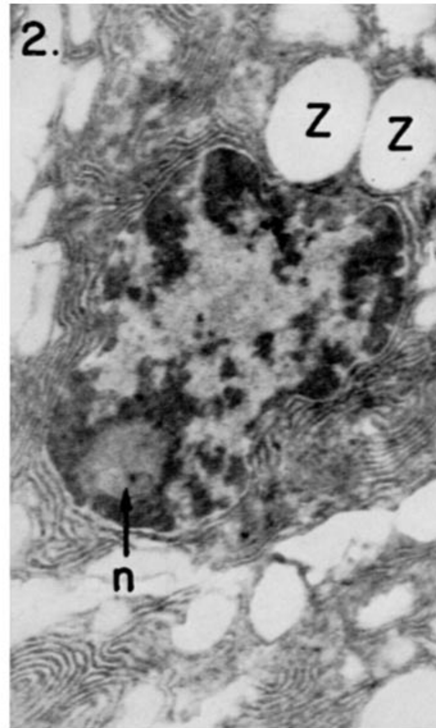

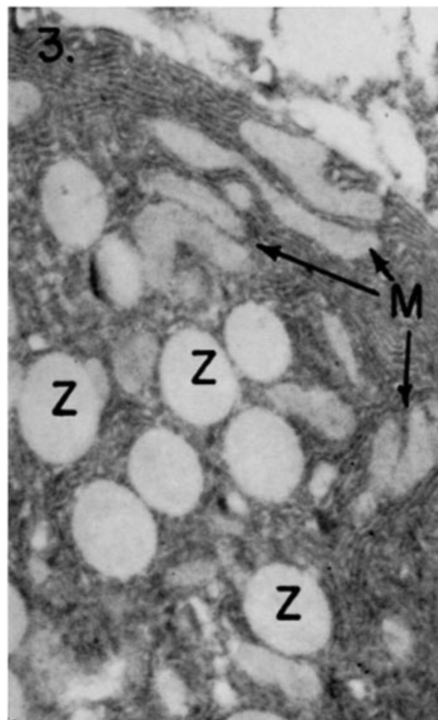

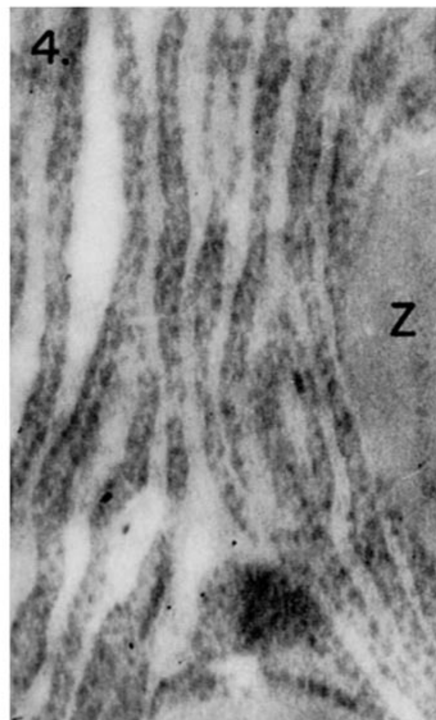

(Low and Freeman: Experiments with chromium compounds as fixers)

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