ELECTRON MICROSCOPY OF THE OXYNTIC CELL IN THE GASTRIC GLANDS OF THE BULLFROG, *RANA CATESBIANA*

III. Permanganate Fixation of the Endoplasmic Reticulum

ALBERT W. SEDAR. From the Daniel Baugh Institute of Anatomy, Jefferson Medical College of Philadelphia

In recent studies (7, 8), the fine structure of the oxyntic cell was found to change during acid secretion. The main element affected was the system of smooth surfaced tubules, cisternae, and vesicles (which represents most of the endoplasmic reticulum of this cell). In many instances, the smooth surfaced elements were found to form a reticulum or a continuous three-dimensional network. However, there was present in the preparations a number of closely apposed circular profiles which apparently correspond to isolated vesicles. In some cases, the discontinuity of the elements was ascertained with serial sections. Although it might be possible for a reticulum to break down temporarily and exist in a dispersed form, it is equally possible that the preservation, with buffered osmium tetroxide, or some other feature of the preparatory procedure was not optimal, causing the reticulum to fragment into vesicles. Therefore, an alternative procedure was used to evaluate the results previously obtained with osmium tetroxide fixation. Permanganate was chosen because of its good preservation of membrane-limited structures although it is unsatisfactory for most of the fibrous and particulate components of the cytoplasm (1, 3). This paper describes the findings and suggests that potassium permanganate fixation coupled with Epon embedding provides adequate preservation for the endoplasmic reticulum in the oxyntic cell.

MATERIAL AND METHODS

Bullfrogs (*Rana catesbiana*), ranging in weight from 150 to 200 grams, were maintained in the laboratory at room temperature. Frogs were pithed and the stomachs exposed and opened by an incision along the greater curvature. The pH of the gastric contents was determined with hydrion papers. Only frogs with gastric contents showing a pH of approximately 7 were used for obtaining tissue specimens.

For electron microscopy, tissue specimens from the corpus of the stomach were fixed for 2 hours at room temperature in 3 per cent $KMnO_4$ (5) buffered with 0.06 M KH_2PO_4-K_2HPO_4 at pH 7.6. The tissue was then dehydrated in ethanol and propylene oxide, and embedded in Epon 812 (4). Sections 600 to 900 A thick (6) were cut from blocks with the LKB Ultrotome and mounted on carbon-coated 150- or 200-mesh copper grids (11).

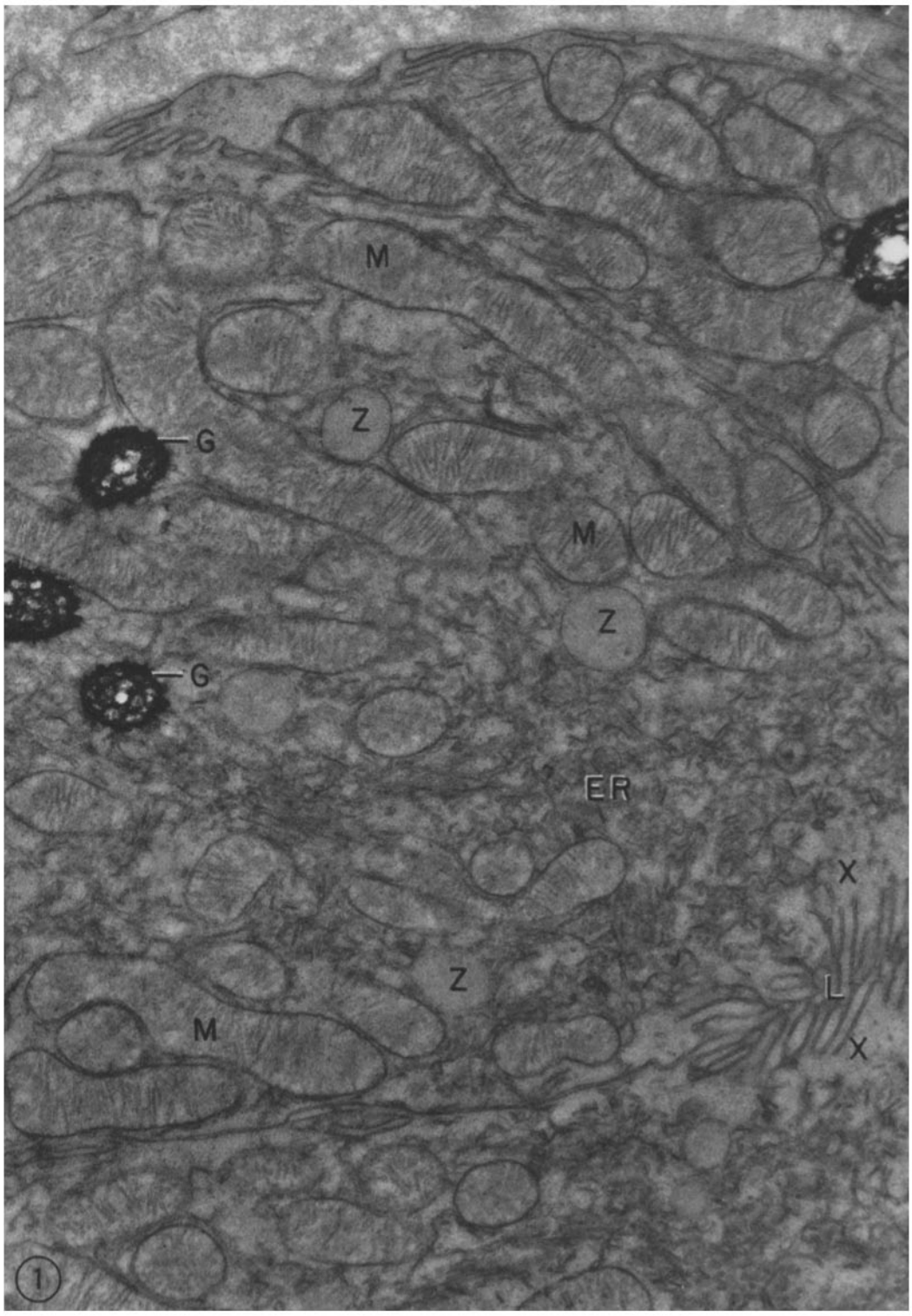
Microscopy was done with an RCA EMU 3D electron microscope containing a 1 mil platinum objective aperture and using an acceleration voltage of approximately 100 kv. Micrographs were taken at original magnifications of 5,400 to 21,800 diameters and enlarged or reduced photographically as required.

OBSERVATIONS AND DISCUSSION

After $KMnO_4$ fixation, the oxyntic cell in the gastric glands of the frog is seen to contain many of the components previously described after OsO_4 fixation in the same and other species (9, 7, 8, 2),

FIGURE 1

Portions of three adjacent oxyntic cells facing an occluded glandular lumen (*L*) are illustrated in the micrograph. Mitochondria (*M*) are numerous, each of which contains a high proportion of closely spaced cristae. Membrane-delimited zymogen granules (*Z*) as well as poorly fixed granules (*G*) of unknown composition are also seen. The apical surface of the cells facing the lumen (*L*) of the gastric gland is differentiated into a number of closely apposed microvilli. The remaining portion of cytoplasm, except for a relatively undifferentiated zone of apical cytoplasm (*X*), is occupied in large part by the tubular and cisternal elements of the endoplasmic reticulum (*ER*). The width of these profiles averages 250 A and this measurement appears remarkably constant in the micrographs. × 18,000.



although ribonucleoprotein particles are not preserved. Mitochondria are especially numerous, each of which possesses a large number of cristae or lamellae (Figs. 1 and 2). The differentiations of the cell surface facing the lumen of the gland are of irregular contour as the consequence of elaborate outpocketings and infoldings of the cell membrane (Figs. 1 and 2); the basal portion of the cell also has complicated infoldings of the plasma membrane. In most instances, as illustrated in the micrographs, the lumina of the gastric glands are occluded. Zymogen granules and poorly fixed granules of unknown composition are also seen. The remaining portion of cytoplasm, except for the relatively undifferentiated zone subjacent to the lumen of the gastric gland, contains a striking array of smooth surfaced elongated profiles. These elements appear to assume a very complex orientation and to form a reticulum or three-dimensional continuum. The width of the profiles averages 250 A. This system is composed of tubular and cisternal (flattened sacs) elements. The cisternae are well illustrated in Fig. 2, and can be compared here with elongated profiles found elsewhere in the micrograph. The volume of this system is large and extends to all areas, showing no preferred orientation in the cell (Figs. 1 and 2). In addition, tubular elements are found open at the cell surface at both poles of the cell (basal and apical). Transcellular continuity is suggested by the findings but remains to be proven. It seems reasonable to identify the bulk of this system as the agranular endoplasmic reticulum since the granular reticulum constitutes only a small percentage in the oxyntic cell of the frog (7).

It is apparent, after comparing the endoplasmic reticulum observed here following permanganate fixation with that previously demonstrated with osmium tetroxide (7), that a greater percentage of elongated profiles and relatively few vesicles are evident after permanganate fixation. Recently, using osmium tetroxide fixation, Ito (2) reported similar findings in amphibian oxyntic cells with modified preparatory procedures. He stressed the rapid passage (5 minutes) of tissue through cold alcohols to absolute ethanol, where the tissue remained for 2 to 3 hours at room temperature, and the use of uranyl nitrate as recommended by Ward (10) in methacrylate embedding. In order to obtain more information on the possible role of the agranular reticulum in the general economy of the oxyntic cell, its fine structure will be re-examined under experimental conditions using $KMnO_4$ fixation.

This study was supported by the United States Public Health Service grant RG-4810 (C5).

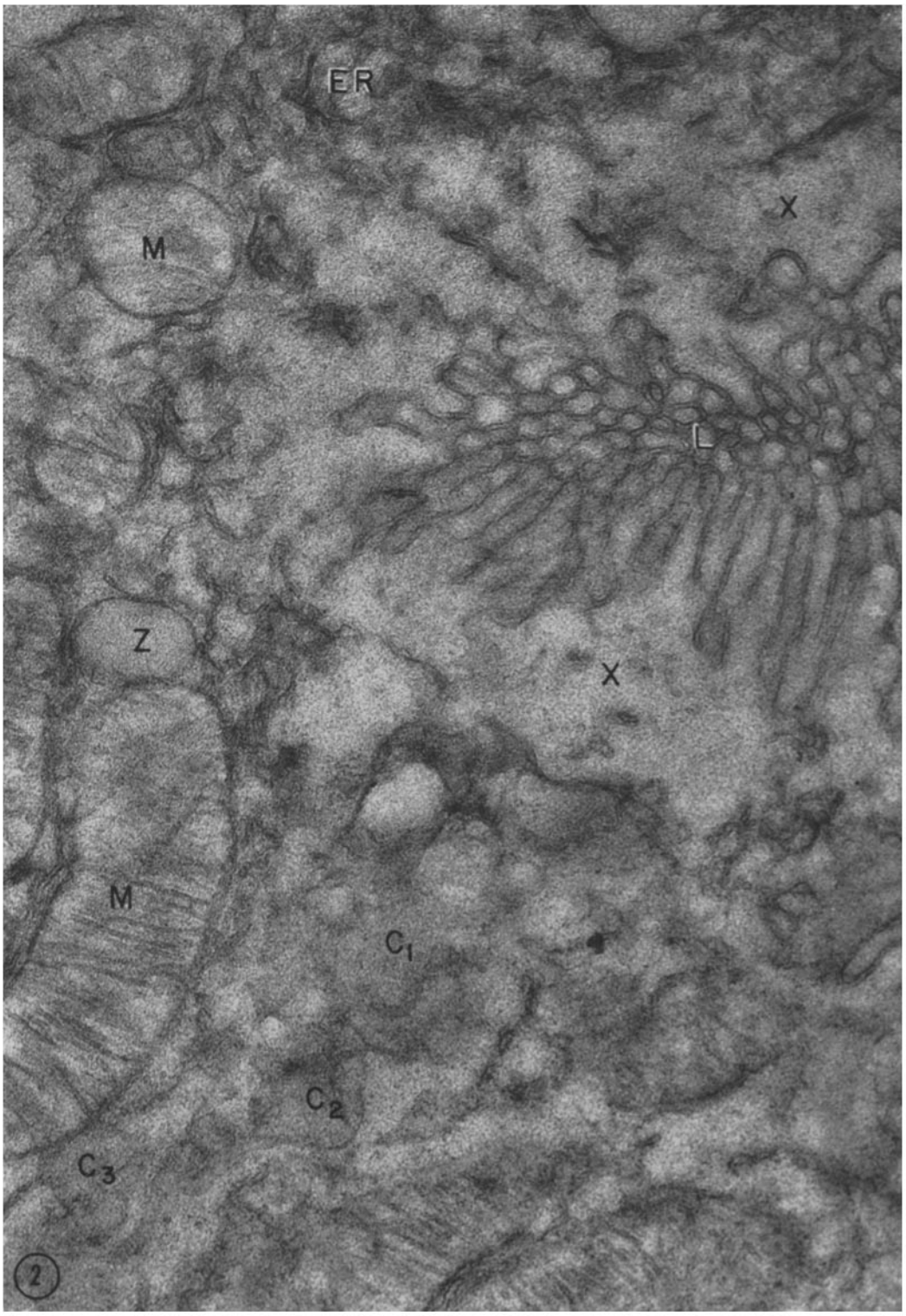
Received for publication, March 3, 1962.

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. ITO, S., The endoplasmic reticulum of gastric parietal cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 333.
3. LUFT, J. H., Permanganate—a new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
4. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
5. MOLLENHAUER, H. H., and ZEBRUN, W., Permanganate fixation of the Golgi complex and other cytoplasmic structures of mammalian testes, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 761.
6. PEACHEY, L. D., Thin sections. I. A study of section thickness and physical distortion produced during microtomy, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 233.

FIGURE 2

Part of an oxyntic cell adjoining an occluded lumen (*L*) of a gastric gland is seen in the micrograph. Mitochondria (*M*) and a zymogen granule (*Z*) are also represented. The apical surface of the cell consists of closely spaced finger-like projections of cytoplasm. Subjacent to these elements there exists a zone (*X*) lacking many formed elements. Much of the rest of the cytoplasm contains tubular and cisternal elements of the endoplasmic reticulum (*ER*). The cisternae are particularly well illustrated at C_1, C_2 and C_3 where these flattened sacs have been sectioned tangentially. These cisternae appear to be confluent. × 36,000.



7. SEDAR, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbiana*). I. The non-acid secreting gastric mucosa, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 1.
8. SEDAR, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbiana*). II. The acid secreting gastric mucosa, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 47.
9. VIAL, J. D., and ORREGO, H., Electron microscope observations on the fine structure of parietal cells, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 367.
10. WARD, R. T., Prevention of polymerization damage in methacrylate embedding media, *J. Histochem. and Cytochem.*, 1958, **6**, 398.
11. WATSON, M. L., The use of carbon films to support tissue sections for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 183.