PATTERN OF OSMIUM DEPOSITION IN THE PARIETAL CELLS OF THE STOMACH

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ABSTRACT

Parietal cells of the stomach of the hamster show extensive amounts of dense material in a variety of organelles after prolonged exposure to a solution of osmium tetroxide. Conspicuous amounts of reduced osmium compounds are evident within the granular endoplasmic reticulum, perinuclear cisterna, and vesicular elements of the Golgi complex. Dense material is also apparent within cristae of the mitochondria, the surface coat of the microvilli of the intracellular canalculus, and vesicular elements of the multivesicular bodies. Multivesicular bodies, containing numerous small osmiophilic elements, are often seen surrounding and/or in close contact with mitochondria. The proximity of the multivesicular bodies to the mitochondria appears to be related to an autophagic process involving degradation of mitochondria. The distribution and intensity of the precipitates within the organelles of the parietal cells vary in different regions of the gastric glands. The findings of this study emphasize that cell structures other than the Golgi complex may contain large concentrations of reduced osmium compounds after prolonged exposure to a solution of osmium tetroxide.

The use of osmium tetroxide as a cytological marker for the Golgi complex in electron microscopy was introduced by Dalton and Felix in 1954 (1). Since that time there have been a number of investigations on a variety of tissues (2-10) using this technique. Although certain elements of the Golgi complex are readily visualized with this method, the perinuclear cisterna and the granular endoplasmic reticulum have also demonstrated conspicuous amounts of dense deposits with this technique (2, 4, 7). More recent studies (8-10) have demonstrated osmium precipitates within mitochondria and other cellular components. Since the presence of reduced osmium compounds in the vesicles of multivesicular bodies appears to be related to both heterophagy (7) and autophagy (10), we were prompted to study the distribution of reduced osmium compounds in relation to the autophagic process of parietal cells (11) in the stomach of hamsters.

MATERIALS AND METHODS

These studies were performed on adult male and female hamsters weighing 100-125 g. Samples of gastric mucosa were taken from the body of the stomach and cut into thin strips. Tissues were immersed in 2% osmium tetroxide solution buffered with 0.07 M s-collidine (pH 7.4) for 20 min at room temperature. After this brief period of fixation, the samples of gastric mucosa were rapidly rinsed in distilled water and then immersed in 2% osmium tetroxide solution. In this solution, the vials of tissue were then placed in a light-sealed oven for 60 h at 38°C. At equal intervals during the 60-h fixation period, the unbuffered osmium tetroxide solution was changed two to three times and replaced with fresh unbuffered osmium tetroxide solution.

After the final fixation step, the tissues were rapidly...
FIGURE 1 Parietal cells from the upper portion of the gastric glands show prominent dense deposits within the mitochondria (M). Most of the mitochondria in this field show heavy depositions; however, several profiles of this organelle show no conspicuous amounts of precipitates. The intense deposits produce a distinct contrast in the parietal cell (P) and the mucous neck cell (N) which shows prominent precipitates in the outer elements of the Golgi complex (G). × 10,500.
rinsed in distilled water and then soaked in an aqueous solution of 0.05% uranyl acetate for 1 h at room temperature. Tissues were dehydrated in cold methanol solutions. After several changes of propylene oxide, small pieces of gastric mucosa were embedded in Epon 812 (12). Sections with silver to gold interference colors were cut on an LKB Ultrotome (LKB Instruments, Inc., Rockville, Md.), mounted on unsupported 300-mesh grids, and stained with lead citrate (13). Electron microscope images were recorded on du Pont Cronar (COS-7) film (E. I. DuPont de Nemours & Co., Wilmington, Del.) with the Siemens Elmiskop IA.

OBSERVATIONS

Morphology

The ultrastructure of the parietal cell of the stomach of the hamster after routine fixation for electron microscopy (11) bears a close resemblance to that of the bat (14). The parietal cell is the largest epithelial cell of the gastric glands. The nucleus occupies the center of the cell. Between the nucleus and the outer limits of the cell is a conspicuous intracellular canaliculus which has a prominent microvillous border. The numerous mitochondria are most abundant in the peripheral and perinuclear cytoplasm. The intracellular canaliculus often appears partially to separate the mitochondria into distinct inner and outer rings.

The mitochondria are large and present round and/or oval profiles which show distinct and closely packed cristae.

The pericanalicular cytoplasm is usually free of mitochondria and possesses numerous smooth-surfaced vesicles which have round and/or slightly irregular profiles. The granular endoplasmic reticulum is usually poorly developed in the mature cells and is confined to the periphery of the cell. In some cases, elongate cisternae are seen extending into the pericanalicular cytoplasm.

Upper Region of the Gastric Glands

MITOCHONDRIA: After extended periods of osmication (60 h) of the gastric mucosa, parietal cells in the upper portion of the gastric glands usually show prominent amounts of reduced osmium compounds only in the mitochondria. The cells nearest the gastric pits show the heaviest amounts of reduced osmium compounds within the mitochondria. At low magnifications (Fig. 1), the deposits are so intense that definition of the individual cristae of the mitochondria is obscured. In this region of the gland, most of the mitochondria show the intense osmium reduction, but some of the mitochondria show only small amounts of the osmium precipitates. At higher magnifications (Figs. 2 and 3), impregnation of osmium is seen in

Figures 2 and 3 The patterns of deposition of dense material in the mitochondria of the parietal cells in the upper portion (Fig. 2) and lower portion (Fig. 3) of the gastric glands are illustrated in these figures. Intense deposition is shown between the outer and inner limiting membranes and in the intracristal space of the mitochondria. In some cases, the double membrane systems appear as solid lines delimiting and traversing the mitochondria due to the heavy concentration of precipitates. Mitochondria of parietal cells in the lower portion of the gastric glands (Fig. 3) show deposits in the same areas as seen in Fig. 2; however, lesser amounts of the precipitate are seen between the membranes which delimit the mitochondria and form the cristae. Fig. 2, × 49,000. Fig. 3, × 44,000.
FIGURE 4 Extensive deposition of dense material is seen in the parietal cell in the center of this field. Most of the accumulations of precipitates are evident within the perinuclear cisterna (P) and cytoplasmic vesicles (V) which intervene between the nucleus and intracellular canalicus (I). Greater detail of the pattern of deposition is seen in Fig. 5. Precipitates are not evident in the chief cells (C) and connective tissue space (T) which surrounds the parietal cells. × 32,500.
relation to the limiting membrane and cristae of the mitochondria. In these regions, precipitates are evident between the outer and inner leaves of the limiting membrane of the mitochondria and the individual membranes which form the cristae. Mitochondria with prominent amounts of dense material show no spaces between the membranes which compose the limiting membrane and the cristae (Fig. 2). Mitochondria with lesser amounts of the deposits exhibit electron-transparent areas alternating with regions of intense precipitation between the membrane systems forming the cristae and the limiting membrane of the mitochondria (Fig. 3). No conspicuous amounts of deposits occur within the homogeneous matrix of the mitochondria.

Lower Region of the Gastric Glands

The pattern of osmium deposition after extended osmication periods in the parietal cells of the lower region of the gastric glands differs from that described above.

PERINUCLEAR CISTERNA AND GRANULAR ENDOPLASMIC RETICULUM: One of the most conspicuous areas of osmium deposition in the parietal cell is the perinuclear cisterna. This is readily appreciated at low magnifications (Fig. 4). The pattern of deposition of the reduced metal compound often appears irregular and the cisterna shows a dilated and discontinuous appearance; this is apparently due to the plane of section of the parietal cell and the continuity of the perinuclear

FIGURE 5 Deposition of dense material is prominent within the perinuclear cisterna ($P$) of the parietal cell. Additional precipitates are present in the small and irregularly shaped vesicles ($V_1$) between the nucleus ($N$) and intracellular canaliculus ($I$). Larger vesicles and smaller ones with smooth contours ($V_2$) in this same region lack the precipitates. Elongate cisternae of the granular endoplasmic reticulum ($G$) also show extensive deposits. These tubular elements are usually found between the intracellular canaliculus and the limiting membrane of the parietal cell. A small multivesicular body ($MV$) shows no deposits. $\times$ 22,000.
cisterna with other organelles. At higher magnifications, more detail of the pattern of the deposition is gained (Fig. 5). The lumen of the cisterna varies from 200 to 400 Å in width and usually contains generous amounts of precipitates; breaks in deposition pattern are apparent at sites of the nuclear pores. Elements of the granular endoplasmic reticulum and irregularly shaped vesicles appear in close contact and/or in continuity with the perinuclear cisterna. The granular endoplasmic reticulum (Fig. 5) shows intense osmium deposition, thereby revealing its distinct profile. This organelle is apparent as irregularly shaped tubular structures of moderate length. The reduced osmium compounds are uniformly distributed throughout the regular and dilated segments. The density and irregular contours of the precipitates within the cisterna often obscure definition of the attached ribosomes on the outer surface of the granular endoplasmic reticulum. Although the granular endoplasmic reticulum is most prominent in the peripheral cytoplasm of the parietal cell, elements of this organelle are often evident in direct continuity with the perinuclear cisterna; cross-sectional profiles of the granular endoplasmic reticulum in this region of the parietal cell are difficult to differentiate from the irregularly shaped pericanalicular vesicles.

Pericanalicular Vesicles and Surface Coat: Prominent accumulations of reduced osmium compounds appear within the pericanalicular cytoplasm of the parietal cells (Figs. 4 and 5). These deposits of the reduced metal compounds are seen within vesicles which are concentrated in the portion of the cytoplasm between the nucleus and the intracellular canaliculus. In the latter area (Fig. 5), there are smooth-contoured and irregularly shaped vesicles. The irregularly shaped vesicles show heavy concentrations of the deposits, while the smooth-contoured

![Figure 6](image)

**Figure 6** Heavy deposition of dense material is seen associated with the intracellular canaliculus (I) of the parietal cell and obscures the microvilli which project into the lumen of the canaliculus. The irregular pattern of deposition within the surface coat of the microvilli gives the intracellular canaliculus a mottled appearance. Parietal cells which reveal extensive deposition in cytoplasmic organelles as shown in Figs. 1-5 usually have no precipitates on the luminal surface of the intracellular canaliculus. × 43,200.  

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vesicles contain no detectable amounts of the precipitates. The smooth-contoured vesicles are usually larger than the irregularly shaped forms; however, there are smaller smooth-contoured vesicles the size of which often approximates that of the individual vesicles of the multivesicular bodies present in the pericanalicular region.

The intracellular canaliculus of the parietal cells has numerous microvilli which project into the lumen of this compartment. The microvilli are closely apposed and/or interdigitate, thereby nearly obliterating the lumen. The free surface of the microvilli has a thin amorphous outer coat which is continuous over the entire microvillous surface of the intracellular canaliculus. The precipitates are evident as small and irregularly shaped profiles of reduced osmium compounds within the surface coat (Fig. 6). Each of the deposits is smaller than the individual precipitates observed within the irregularly shaped vesicles present in the pericanalicular area described above. The deposits show a random distribution within the surface coat. The presence of intense osmium deposition was not observed in the surface coat of all parietal cells; however, the precipitates were usually more prominent in cells which showed sparse osmium deposition in cytoplasmic organelles of the parietal cells.

GOLGI COMPLEX AND MULTIVESICULAR BODIES: The Golgi complex is a poorly developed organelle in the parietal cells of the hamster. This structure is usually observed in the periphery of the cell in close proximity to the limiting membrane. The Golgi complex (Fig. 7) is composed of outer and inner elements; however, the latter elements differ in appearance from those seen in other cells. Instead of consisting of long, flattened cisternae with convex and concave faces, the inner portion is evident as large elongate vesicles which appear to interconnect with one another. The outer portion is apparent as numerous small vesicles which appear arranged in rows in close proximity to the inner portion. Intense osmium deposition occurs within the smaller vesicles of the outer portion; no osmium precipitates were observed within the large vesicles of the inner region of the Golgi complex.

Parietal cells contain a moderate number of multivesicular bodies which are randomly distributed throughout the cytoplasm. These organelles typically appear as circular, membrane-bounded structures containing small vesicular elements. Most of the profiles of the multivesicular bodies are comparable in diameter to those of the mitochondria; however, many appear about 0.5 this dimension. After prolonged exposure to osmium, the large form of the multivesicular bodies shows intense osmium deposition. These precipitates appear to coincide with the small individual vesicles which populate the interior of this organelle (Figs. 8–10). The smaller multivesicular bodies usually show no osmium deposition. The large multivesicular bodies are frequently seen in association with mitochondria. In many cases, the limiting membrane of the multivesicular bodies is seen in close contact with (Fig. 8), partially surrounding (Fig. 9), and totally surrounding (Fig. 10) mitochondria.

DISCUSSION

The present study has shown that organelles other than the Golgi complex (1) display prominent amounts of dense precipitates after prolonged exposure to a solution of osmium tetroxide. Furthermore, the pattern of osmium deposition differs in parietal cells found in the upper and lower portions of the gastric glands.

It is clear from this study that parietal cells show more prominent amounts of osmium deposition than other epithelial cells of the gastric glands. A
This series of micrographs shows what appears to represent progressive stages of an autophagic process related to the degradation of mitochondria.

**Figure 8** Three multivesicular bodies are present in this field. A small multivesicular body (MV₁) shows no dense deposits. A large multivesicular body (MV₂) has numerous small accumulations of intense precipitation which appears to coincide with individual vesicular elements of the organelle. Several of the small vesicles (unlabeled arrows) show no precipitates. A third membrane-bounded structure, presumably another multivesicular body (MV₃), is seen in close contact with a mitochondrion (M). The accumulations of dense material are concentrated in the periphery of the organelle. × 52,000.

**Figure 9** A multivesicular body (MV) partially surrounds a profile of a mitochondrion (M). The limiting membrane of the multivesicular body is in close contact with the limiting membrane of the mitochondrion. Precipitates are observed within the interior and in close association with the limiting membrane of the multivesicular body. × 52,000.

**Figure 10** A ring of heavy deposition of dense material (arrows) surrounds a mitochondrion (M). The precipitates are apparently contained within a saccular membrane system which totally surrounds and isolates the mitochondrion from the cytoplasmic matrix. The saccular (surrounding) membrane system appears to represent a multivesicular body as shown in Fig. 9. The limiting membrane of the multivesicular body apparently flattens and/or elongates, thereby puckering the irregular masses of precipitates and eventually surrounding the mitochondrion. × 68,000.
The precipitates are slowly produced more or less tivesicular bodies of the parietal cells show intense chondria. Individual smooth vesicles within multivesicular bodies of the parietal cells. Furthermore, the materials, which present study concerns the multivesicular bodies with the fixative solution at elevated temperatures. React with the components of the fixative solution soaking process; only more of these materials react and produce the precipitates, are in all likelihood throughout the tissue during the soaking process and diffuse to a variety of different sites in the parietal cells. It is tempting to speculate that certain enzymes localized on the cristae of mitochondria and within certain organelles of the parietal cells, such as the perinuclear cisterna, the granular endoplasmic reticulum, and Golgi complex, are capable of reacting with osmium tetroxide and producing the dense precipitates. However, this does not appear likely, since other workers (15) have shown that most SH groups are destroyed on contact with oxidizing agents such as osmium tetroxide.

Although it is likely that many, if not all, of the dense deposits shown in this study are osmium compounds, there is no evidence presented here to support this speculation. However, it is certain that the dense deposits do not result solely or in part from staining with uranyl acetate or lead citrate, since unstained sections show the same pattern of dense deposits as the sections that were stained; staining with these compounds only improves the contrast of the sections. In addition, the site of dense deposition may not necessarily be the site of reduction. For example, the sites of deposition may contain materials which have a high affinity for the osmium compounds in the fixative solution and result in the formation of dense precipitates. The precipitates are slowly produced more or less throughout the tissue during the soaking process and diffuse to a variety of different sites in the parietal cells. Furthermore, the materials, which react with the components of the fixative solution and produce the precipitates, are in all likelihood unspecific at various temperatures during the soaking process; only more of these materials react with the fixative solution at elevated temperatures.

One of the more intriguing observations in the present study concerns the multivesicular bodies and their relationship to the autophagy of mitochondria. Individual smooth vesicles within multivesicular bodies of the parietal cells show intense osmium deposition similar to that seen in multivesicular bodies of epididymal epithelial cells (7). In the sequence of stages indicated in the autophagic process in the present study, the multivesicular body, i.e., the limiting membrane of the multivesicular body, surrounds and eventually sequesters the mitochondrion from the cytoplasmic matrix. This is apparently related to the process of degradation of mitochondria. Such a process of autophagy involving mitochondria has been demonstrated in parietal cells of the hamster (11) and other cell systems (16, 17). Since osmium tetroxide destroys enzyme activity (15), it appears that the osmium black material within the limiting membrane of the multivesicular body is not a product of acid phosphatase activity (16). However, there is little doubt that such surrounding membrane systems are related to mitochondria which show acid phosphatase activity (11).

Although no definitive explanation for the intense osmium deposition within the variety of cytoplasmic organelles of the parietal cells has been concluded from the present studies, it would not be unreasonable for a cell which displays high metabolic activity, such as a parietal cell, to show intense osmium deposition without regard to the specific chemical composition of the material found in the osmium black areas. Any absolute identification of the material producing the intense deposition will require correlated biochemical analysis.

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