Cytotoxic Effects of D-Glucosamine on the Ultrastructures of Normal and Neoplastic Tissues in Vivo

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SUMMARY

The effects of D-glucosamine on the fine structures of Walker carcinoma and of the liver and kidney in rats were studied in vivo.

Continuous administration of D-glucosamine for 18 hr resulted in a slight dilation of the cisternae of the endoplasmic reticulum and Golgi sacs of the tumor cells and liver parenchymal cells. The epithelial cells of the renal tubules contained lamellar structures within numerous autophagic vacuoles.

Infusion of D-glucosamine for 40 hr resulted in conspicuous degenerative changes in the nuclei and nucleoli of the Walker tumor cells. The nuclear matrix showed a much-decreased electron density and contained clumped interchromatin granules. The nucleoli had rounded up, and the strands of the nucleolonema had coalesced. Conspicuous small aggregates of high contrast, probably representing chromatin, had formed predominantly in the electron-lucent areas of the nucleolus. Cytoplasmic changes in tumor cells were relatively slight. The parenchymal cells of the liver showed almost complete fragmentation of the long profiles of the rough endoplasmic reticulum into small vesicles. Only single rows of the usual staggered array of cisternae remained surrounding mitochondria. Autophagic vacuoles were frequently seen. The lining epithelium of the proximal convoluted tubules of the kidney showed striking vesiculation of the cytoplasm. At no time was necrosis of the renal or hepatic cells observed.

The most significant finding was the complete necrosis of tumor cells in rats sacrificed 5 days after 40 hr of infusion with D-glucosamine. This was accompanied by a remarkable recovery of the fine structure of the renal and hepatic parenchymal cells.

INTRODUCTION

The inhibitory action of D-glucosamine and other sugar analogs on the viability and transplantability of Ehrlich ascites carcinoma and Sarcoma 37 and Sarcoma 180 ascites tumors was reported previously (4). Electron microscope study has shown that D-glucosamine provoked irreversible ultrastructural changes in these tumor lines, changes that were not apparent when the incubation was carried out in the presence of D-glucose (22, 23).

Since the original observations made by Quastel and Cantero (25), D-glucosamine has been repeatedly tested as an antitumor agent (2, 17, 40). More recently, exogenous D-glucosamine has been shown to inhibit the growth of various experimental tumors in vivo (6) and selectively to inhibit the biosynthesis of protein, RNA, and DNA of neoplastic tissue in vitro and in vivo (3, 5—7).

These observations made it desirable to carry out cytological examinations of tumor tissue obtained from rats bearing Walker 256 carcinoma during and after glucosamine treatment and compare the cytotoxic effect to that of glucosamine on host organs. The present study deals with the ultrastructure of tumor, liver, and kidney cells after i.v. glucosamine treatment.

MATERIALS AND METHODS

Animals. Adult, male, Sprague-Dawley rats weighing 310 to 360 g were used. The animals were maintained in a thermostatically controlled room at 20° with 12 hr of light in the cycle. They were fed Purina chow and tap water ad libitum. Transplantation of Walker 256 carcinoma was carried out as described previously (6).

Treatment. Rats bearing 6- to 7-day-old i.m. Walker tumors measuring 10 to 20 mm in diameter were used for this study. The tail vein of the rat was cannulated, and then the tubing was attached to a Harvard syringe type infusion pump. A 0.75 M glucosamine solution was infused continuously at a rate of 0.8 to 0.9 ml/hr, which corresponds to about 350 mg/kg/hr. Details of this procedure are described in a separate paper (6).

Both treated and control animals were kept in a plastic restrainer.

Tissue samples were obtained from the tumors, kidneys, and livers of 2 rats before infusion with glucosamine, after 18 and 40 hr of infusion, and 5 days after the end of 40 hr of infusion. For electron microscopy, specimens were fixed in cold 4% paraformaldehyde buffered with s-collidine, pH 7.4, containing 0.005 M calcium chloride, and were postfixed in 2% osmium tetroxide that was buffered with s-collidine at pH 7.4 (16). After fixation, the tissues were dehydrated and were embedded in Epon 812 resin. For light microscopy, sections approximately 1 μm thick were cut and stained with Mallory's azure 2-methylene blue. Thin sections were cut with diamond knives on Sorvall MT-2 or Reichert OM U2 ultramicrotomes.
Sections were stained with lead citrate and uranyl acetate. Specimens were studied on a Philips EM 200 electron microscope, and photographs were made on Eastman Kodak electron image plates.

RESULTS

Light Microscopy

When the differences in preparation are taken into account, the Walker carcinoma tumors from i.m. sites in this study resembled Walker tumors described previously (10, 13). In the present preparations, the cytoplasm of large tumor cells varied appreciably in staining intensity (Fig. 1). The vacuolated tumor cells described by Earle (10) were not observed, perhaps because of the difference in the age of the tumor cells examined. Infusion of D-glucosamine for 18 or 40 hr resulted in no immediate noticeable alteration in the tumor cells. However, 5 days after 40 hr of infusion with D-glucosamine, the entire tumor mass consisted of noncohesive, necrotic tumor cells (Fig. 2). No intact tumor cells were recognized.

After 18 hr of infusion with D-glucosamine, the parenchymal cells of the liver showed no change in relation to untreated livers. Infusion for 40 hr resulted in loss of the usual tinctorial pattern of the parenchymal cells. The majority of the cells showed greatly decreased staining intensity attributable to loss of stacks of the RER2. The cytoplasm of some cells appeared evenly, darkly stained, probably because of decreased hydration. Most liver cells contained a variable number of small, clear vacuoles (Fig. 3). The nucleoli were conspicuously enlarged. Parenchymal cell necrosis or inflammatory infiltrates, observed on administration of D-galactosamine (15), were not seen in the present study. Five days after 40 hr of infusion, sinusoids and vessels of the liver were dilated, but the parenchymal cells of the liver showed no apparent light microscopic change in relation to untreated cells. Kupffer cells were occasionally prominent in the widened sinusoids.

The renal tubular epithelium and glomeruli of rats that were infused for 18 hr showed no change in relation to untreated kidneys. Infusion for 40 hr resulted in vacuolization of the proximal tubular epithelium. The incidence and degree of vacuolization varied, as shown in Fig. 4. Glomeruli and tubules of the medulla showed no change. Five days after 40 hr of infusion, the tubules of the renal cortex demonstrated no change in relation to untreated rats. Necrosis or mitosis of the renal and hepatic parenchymal cells was not observed.

Electron Microscopy

Fine Structure of Walker Carcinoma and Liver and Kidney Cells from Untreated Rats. The appearance of the fine structure of Walker carcinoma cells that were fixed with paraformaldehyde was essentially as described for Walker tumor cells from s.c. or hepatic sites in earlier studies (13, 21, 24, 31). In most cells, the nucleus was excentric and the cytoplasmic organelles were polarized on 1 side of the nucleus. The number of cytoplasmic organelles greatly varied in the tumor cells: some apparently primitive, electron-lucent cells with a small cytoplasm contained very few organelles. The more numerous large tumor cells showed abundant mitochondria, RER, ER, and a well-developed Golgi apparatus (Fig. 5). The electron density of the tumor cells varied appreciably: it seemed to depend on their state of hydration and on the number of cytoplasmic organelles, particularly on the amount of fine cytoplasmic filaments, which measured, on the average, 90 Å in diameter.

In contrast to previously reported findings (13, 21, 24, 31), small, desmosome-like junctions were frequently seen between adjacent tumor cells (Fig. 6). Unlike the results obtained by Smeta and Busch (31) with formalin-fixed cells, chromatin of nuclei, including nucleolus-associated chromatin of untreated tumor cells, was inconspicuous and evenly distributed in the present study. Clumping of interchromatin was not observed in untreated tumor cells. In agreement with previous reports (32), the nucleoli showed rather loosely woven strands of the nucleolonema, consisting of the peripheral granular part and the mixed granular-filamentous, electron-dense nucleolonema, which surrounded small fenestrations of low electron density (Fig. 10). “Purpure filamentous parts were scanty, as was reported earlier (32). Electron-dense granules in the nucleolus (27) were not observed in the present study.

The fine structure of the parenchymal liver cells of untreated, tumor-bearing rats appeared essentially as described for nontumor-bearing rats (8). Small lamellar structures were occasionally seen in the cytoplasm. Glycogen was not visualized, probably because the rats were fasting and a fixative buffered with s-collidine was used. The fine structure of renal proximal tubular cells of untreated tumor-bearing rats showed no essential difference from previous descriptions (18).

Fine Structural Changes of Walker Carcinoma and Liver, and Kidney Cells of Rats after 18 Hr of Infusion with D-Glucosamine. Golgi vesicles, sacs, and cisternae of the ER were widened in all Walker carcinoma cells that were examined (Fig. 7), but mitochondria appeared unaltered. In a large number of tumor cells, the electron-dense, mixed granular-filamentous part of the nucleolus was more conspicuous (more electron opaque) than in untreated tumor cells (Fig. 11). The nuclear matrix showed no change. The liver cells (Fig. 13) showed vesiculation of the terminal portions of the cisternae of the ER and Golgi vesicles. Mitochondria with bizarre, elongated shapes were found in most cells examined. The matrix of mitochondria was electron dense. The lining epithelial cells of the proximal tubules of the kidney contained many large, swollen mitochondria and numerous, large, autophagic vacuoles. The vacuoles that contained floccular-filamentous matter appeared to be extremely dilated ER or RER; others were filled with irregular lamellar fragments of lipoprotein membranes (Fig. 17). Interstitial cells, glomeruli, and the epithelium of the tubules of the medulla showed no change.

Fine Structure of Walker Carcinoma and Liver, and Kidney Cells of Rats after 40 Hr of Infusion with D-Glucosamine. The

2The abbreviations used are: RER, rough endoplasmic reticulum; ER, smooth endoplasmic reticulum.
most conspicuous change in Walker carcinoma cells was the pronounced alteration in the nuclei and nucleoli. The nuclear chromatin was clumped along the nuclear membrane, and interchromatin granules were aggregated in the rarefied nuclear matrix (Fig. 8). Nearly all nucleoli were rounded up. The previously loosely winding strands of the nucleolonema were tightly compacted and coalesced in all nucleoli examined. At the same time, many small, highly contrasted aggregates appeared mainly within the spaces of low electron density in the nucleolus (Fig. 12). These aggregates closely resembled in consistency and electron density the clumps of interchromatin of the nuclear matrix. The previously inconspicuous, nucleolus-associated chromatin was prominent. Unlike the results obtained on in vitro administration of D-glucosamine (23), separation or extrusion of the electron-lucent components of the nucleolus was not observed. Cytoplasmic changes were less pronounced than the nuclear alterations; occasional mitochondria showed focal swelling with loss of cristae, and cisternae of the ER were focally dilated. In some cells, small accumulations of multivesicular bodies were seen, usually in the peripheral cytoplasm.

The cytoplasm of all parenchymal liver cells examined in the electron microscope showed degenerative changes (Fig. 14): the stacks of long cisternae of the RER were entirely absent. Instead, small vesicles with focally attached ribonucleoprotein particles were abundant. Only single, long cisternae of the RER remained, in close relationship with mitochondria. Smooth portions of the rough vesicles and denuded segments of the retained long profiles of the RER formed closer contacts with the mitochondrial outer membrane (9). In a part of the mitochondrial population, the matrix was more electron dense than usual (Fig. 14), and slight focal widening of the intracisternal space was seen in some mitochondria (Fig. 15). Single, membrane-limited bodies containing heterogeneous material (autophagic vacuoles) were numerous. Increase in the number of the ER vesicles or change in the Golgi apparatus were not noted. Nuclei and nucleoli showed no change. As was seen in light microscopy, darker cells were intermingled with the cells that showed a cytoplasm of the usual density (Fig. 15). These cells also showed the changes already described; in addition, they showed tightly packed organelles in a scanty cytoplasmic matrix. Their nuclei showed multiple shallow indentations. Bile canaliculi, sinusoidal spaces, and Kupffer cells showed no change.

The epithelial cells of the renal proximal tubules (Fig. 18) showed pronounced ballooning of the cytoplasm because of abundant, large, single membrane-limited vesicles. These appeared "empty" or contained a scanty amount of finely filamentous-flocculent material. The mitochondria showed moderate alteration in their cristae, with focal breakdown of their membranes. Microvilli were focally absent, which may represent an artifact produced in a cell that is susceptible to injury. The lumina of the tubules contained much cell debris. As in the altered liver cells, nuclei and nucleoli showed no change in fine structure. The tubular epithelial cells of the medulla, the glomerular epithelium, and mesangial cells showed no significant alteration.

Fine Structure of Walker Carcinoma and Liver and Kidney Cells 5 Days after the End of 40 Hr of Infusion with D-Glucosamine. Infusion of glucosamine resulted in complete necrosis of the Walker carcinoma tumor cell population 5 days after termination of treatment. The entire tumor mass consisted of noncohesive, "ghost-like," interrupted, lipo-protein membrane outlines of tumor cells (Fig. 9). Nucleoli appeared as rounded masses consisting of the disintegrating filamentous part of the nucleolonema intermixed with highly electron-dense aggregates. The granular part was not recognizable in any of the nucleoli examined.

The parenchymal liver cells (Fig. 16) showed slightly increased vesiculation of the terminal portions of the RER in comparison with the livers of untreated rats, but the stacks of cisternae were again abundantly present. There were many small, lamellar, cytoplasmic structures, particularly in the areas of vesiculation. Large, bilobed mitochondria were numerous. Kupffer cells contained many phagosomes. Sinusoids were patent, but bile canaliculi showed no change.

The renal proximal tubular epithelium (Fig. 19) showed an almost complete recovery of the usual architecture. Vacuolization was absent, and only slight focal dilation of the endoplasmic reticulum was seen, with a fine filamentous content in the cisternae. Microbodies, cytoplasmic dense bodies, and autophagic vacuoles were numerous. Many mitochondria showed bilobed "dumbbell" shapes. Small lamellar structures were frequently overlying mitochondria, the outer nuclear membrane, and membranes of the ER.

DISCUSSION

The present study of fine structure, in good agreement with biochemical (3, 5–7), viability, and transplantation studies (4), shows that D-glucosamine, under certain conditions, causes complete necrosis of well-established Walker carcinoma. Of the 2 groups examined, the 1 that gave the best results was the group of rats that received D-glucosamine for 40 hr. In these animals, the treatment was sufficient to eradicate the tumor cells completely without causing irreversible cytotoxic effects on parenchymal cells. Infusion of D-glucosamine for 18 hr resulted in nonlethal damage to tumor cells.

At the end of 40 hr of infusion, the change in the appearance of the nuclei and nucleoli of tumor cells resembled the early effects of antinomycin treatment (26, 29, 30). The formation of highly contrasty aggregates in the electron-lucent parts of the nucleoli and their resemblance to the clumps of interchromatin granules in the nuclear matrix suggest that D-glucosamine, like actinomycin (26), has a condensing effect on chromatin. Complete "nucleolar segregation," as was observed on prolonged treatment with actinomycin (26, 30) and various other agents (30), was not observed in the present study.

Separation and extrusion of the electron-lucent parts of the nucleoli, which were part of the conspicuous nucleolar changes in the in vitro study with D-glucosamine (23), did not occur in the present in vivo study. The nucleoli of tumor cells that were examined 5 days after 40 hr of infusion with D-glucosamine were rounded and homogeneous, concomitant with severe cytoplasmic changes. This indicated complete cell necrosis, as was described in a study of autolysis (38).

The change in the appearance of the tumor cells at the end of 40 hr of infusion with glucosamine was accompanied by a
conspicuous breakdown of the RER of the parenchymal liver cells. This was interpreted as autolysis in earlier studies of necrosis in vitro (37); unlike the investigation in those earlier studies, however, we found no significant degenerative alteration in the mitochondrial fine structure. Similar changes, with vesiculation of the RER, have been seen in a great variety of conditions (33, 34); but, in most of those other conditions, proliferation of the ER was usually an important additional component of the vesiculation. This is not the case in the present study where the vesicles were clearly fragmented RER, since most still showed partially attached ribonucleoprotein particles.

The changes in the parenchymal liver cells in this study closely resembled the findings in rats fed a protein-deficient diet (11). Mitochondrial “irregular cavities,” described as focal widenings of the intracisternal space in the present report, and wrapping of the RER around mitochondria, were also features in the protein-deficient rats (11). In contrast, the finger-like projections of the RER into its own cisternae, reported in 1 study of the effects of a low-protein diet (33), and the prominent increase in lipid droplets in the other study (11), were not observed in the present investigation. Elongated and irregularly shaped mitochondria, observed after infusion with D-glucosamine, have also been seen in increased numbers in other conditions (20, 28). After D-galactosamine treatment (20), they accompanied conspicuous necrotizing changes in parenchymal cells.

The pronounced cytoplasmic vacuolization of the proximal tubular epithelium was indistinguishable from that reported in earlier descriptions of the effects of hypertonics sugars on renal tubular epithelium (19, 39). These seemingly severe changes were shown to be reversible, as is the case in our study, in which, 5 days after the end of 40 hr of infusion with D-glucosamine, the renal tubular cells showed a nearly normal ultrastructural appearance. The reversibility may be explained by the absence of nuclear and nucleolar change in parenchymal liver and kidney cells at the end of the 40 hr of infusion.

Small, cytoplasmic, lamellar structures were seen in tissues of untreated tumor-bearing rats in this study. They were more frequently seen when they were associated with the endoplasmic reticulum, the mitochondrial outer membrane, and the outer nuclear envelope in tissues of rats treated with D-glucosamine. They were particularly numerous in the restituting renal epithelial cells 5 days after the end of 40 hr of infusion with D-glucosamine. They may represent collapsed vesicles of the ER or they may be artifacts of fixation involving recently synthesized vulnerable portions of the cytoplasmic and mitochondrial membranes.

Similar focal damage of cell membranes and mitochondrial cristae was observed after freezing and thawing of liver cells (36) and as a cytostatic effect of sugar-alcohol derivatives on tissue in Shay’s chloroleukemia (14). The possibility that these irregularities in the lipoprotein membranes represent artifacts of aldehyde fixation was suggested by Ericsson and Biberfeld (12); however, similar small myelin-like figures have been reported to occur within mitochondria of liver cells of riboflavin-deficient animals (35) and in liver cells of rats breathing pure oxygen (28), the tissues in both studies having been fixed with osmium tetroxide. In the restituting liver cells in the present study, we did not observe the highly regularly arranged whorls of membranes that were reported to occur in regenerating liver cells after phenobarbital administration (1). This may be explained by the prominent proliferation of the ER in phenobarbital treatment, which may have served as a possible source for the formation of the membrane whorls.

Because of their presence in such a wide variety of conditions, all of the observed changes in the parenchymal cells following infusion of D-glucosamine are best considered nonspecific. The alterations are reversible in the renal and hepatic cells when, at the same time, D-glucosamine causes complete necrosis of the tumor cells.

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Figs. 1 to 4. Light micrographs of Epon sections stained with Mallory's azure 2-methylene blue. X 6,400.

Fig. 1. Light micrograph of untreated Walker carcinoma shows variation in the staining intensity of tightly packed tumor cells. Nuclei of tumor cells are large, and nucleoli are prominent.

Fig. 2. Walker carcinoma at 5 days after the end of infusion of D-glucosamine for 40 hr indicates necrosis of the entire tissue. Tumor cells are separated and are disintegrating, as shown by the pyknotic nuclei and the ill-defined condensed cytoplasm.

Fig. 3. Portion of the liver of rats at the end of infusion with D-glucosamine for 40 hr shows dark- and light-staining hepatocytes containing many vacuoles. The absence of cytoplasmic basophilia (loss of stacks of cisternae of RER) can be better appreciated in the more lightly stained cells. Nuclei are large and show prominent nucleoli (arrows).

Fig. 4. Section of the renal cortex at the end of infusion of D-glucosamine for 40 hr indicates a variable degree of vacuolization of the epithelial cells; some cells appear spared, but others are balloononed (arrow). The lumina of some tubules contain cell debris.

Figs. 5 to 19. Electron micrographs of paraformaldehyde-fixed tissues that were postfixed with osmium tetroxide. The sections were stained with uranyl acetate and lead hydroxide.

Fig. 5. A portion of an untreated Walker carcinoma cell with many mitochondria, numerous cisternae of the RER, and scattered fine filaments (arrows) around the Golgi zone (G). A small segment of the nucleus (N) shows evenly distributed chromatin. X 22,750.

Fig. 6. Cell surfaces of 2 adjacent Walker carcinoma cells from an untreated rat show small, desmosome-like junctions (arrows). X 38,700.

Fig. 7. Portion of a Walker carcinoma cell at the end of 18 hr of infusion with D-glucosamine shows dilated Golgi sacs (G). Mitochondria (M) and nucleus (N) are intact. X 22,750.

Fig. 8. Walker carcinoma cell at the end of 40 hr of infusion with D-glucosamine. The nucleolus (N) is highly electron lucent, and the nuclear chromatin (C) is clumped near the inner nuclear membrane. The cytoplasm contains dilated, smooth vesicles. Some mitochondria appear unchanged, but others show focal loss of cristae (M). Fine cytoplasmic filaments (F) are abundant. X 22,750.

Fig. 9. Small portion of Walker carcinoma cell representative of tumor cells 5 days after 40 hr of infusion with D-glucosamine shows necrosis of all cell components. Only a "ghost" outline of cell organelles remains. The nuclear matrix (N) and chromatin have disintegrated, and the nuclear envelopes have been disrupted (arrowheads). The nucleolus is homogeneous and devoid of the granular component. The electron dense aggregates probably represent clumped nucleolar chromatin (arrow) and interchromatin in the nuclear matrix (small arrows). X 22,750.

Fig. 10. Nucleolus of untreated Walker carcinoma cell exhibits loosely woven strands of the nucleoloneema with the granular part (g) and mixed granular-filamentous part (between arrows) separated by electron-lucent compartments. X 32,500.

Fig. 11. Small segment of the nucleolus of a Walker carcinoma cell at the end of 18 hr of infusion with D-glucosamine indicates prominent, electron-dense, granular-filamentous component of the nucleoloneema (between arrows). X 32,500.

Fig. 12. Nucleus of Walker carcinoma cell 5 days after the end of 40 hr of infusion with D-glucosamine is representative of all tumor cells examined. Bands of the mixed granular-filamentous nucleoloneema became confluent (between small arrows) and appear less granular. Aggregates of high contrast, presumably clumped chromatin, formed predominantly in the electron-lucent compartments (large arrows). The nucleolus-associated chromatin (c) became conspicuous, and clumps of interchromatin granules (arrowheads) are present in the nuclear matrix. X 32,500.

Fig. 13. Parenchymal liver cell from a rat bearing a Walker 256 carcinoma at the end of 18 hr of infusion with D-glucosamine. The nucleus (N) is unchanged. Golgi sacs (G) are dilated. Mitochondria show an electron-opaque matrix, and some are elongated, with bizarre shapes (M). Microbody (mb) is visible. X 22,750.

Figs. 14 and 15. Parenchymal liver cells from a rat bearing a Walker 256 carcinoma, after 40 hr of infusion with D-glucosamine. The stacks of the RER are absent, and cisternae are fragmented, forming abundant rough vesicles. Only single, long cisternae remained around mitochondria. Numerous autophagic vacuoles (V) with a heterogeneous content are visible, some containing mitochondria (large arrows). The electron-dense cell present in the lower half of Fig. 15 displays crowding of mitochondria and autophagic vacuoles (V). Some mitochondria in all 3 cells show small focal dilations of the intracristal space (small arrows). Microbodies (mb) are present. X 22,750.

Fig. 16. Parenchymal liver cell from a rat bearing a Walker 256 carcinoma, 5 days after the end of infusion with D-glucosamine for 40 hr. The RER again shows stacks of long cisternae, although some vesiculation is still evident. Many large, bilobed mitochondria (M) are visible. Small lamellar structures (arrows) are associated with mitochondrial membranes and the RER.

Fig. 17. Portion of epithelial cell of proximal convoluted tubule from a rat bearing Walker 256 carcinoma at the end of 18 hr of infusion with D-glucosamine. The cytoplasm contains swollen mitochondria (M) and autophagic vacuoles with a heterogeneous content; some vacuoles show predominantly lamellar material (arrows), and others appear to be dilated ER or RER with a filamentous-flocculent content (arrowheads). X 22,750.

Fig. 18. Portion of epithelial cell of proximal convoluted tubule from a rat bearing Walker 256 carcinoma at the end of 40 hr of infusion with D-glucosamine. Large numbers of vacuoles are present in the hydropic cytoplasm: some show a loose filamentous content (V), and others are "empty". Micrrolivary are focally absent (arrows), and the tubular lumen (L) contains much debris.

Fig. 19. Epithelial cell of proximal convoluted tubule from a rat bearing Walker 256 carcinoma 5 days after the end of infusion with D-glucosamine for 40 hr. Cytoplasm near cell surface contains numerous autophagic vacuoles with a heterogeneous content (arrowheads). Many small, lamellar structures (arrows) formed over membranes of cisternae, outer nuclear envelope, and mitochondria. Bilobed mitochondria (M) are present. X 22,750.
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