Ultrastructural Pathology

The Comparative Cellular Basis of Disease

Norman F. Cheville
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SECOND EDITION

Norman F. Cheville, DVM, PhD, DHC
To five scientists who had great impact on my scientific development:

George Christensen—Anatomist, Iowa State University
Barney Easterday—Virologist, Army Biological Laboratories
Harley Moon—Pathologist, National Animal Disease Center
Carl Olson Jr.—Pathologist, University of Wisconsin
Gabriele Zu Rhein—Neuropathologist, University of Wisconsin

... and to the many graduate students and visiting scientists in my laboratory from 1963 to 2004 who contributed electron micrographs, ideas, and new concepts in science:

Mark Ackermann
Timothy Anderson
Lawrence Arp
Juan Badiola
Dean Barnett
Jeanne Barnett
Timothy Bertram
Carole Bolin
Arliss Boothe
Dominique Brees
Freddy Coignoul
Randall Cutlip
Phillipe Detilleux
Mark Dominick
Paul Estes
Olaf Hedstrom
Johnny Hoskins
John Kluge
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Preface

*Ultrastructural Pathology* was originally designed for pathologists who interpret cellular changes in lesions encountered in the postmortem room. This edition has shifted to an *atlas format*, reducing text and grouping and labeling electron micrographs for easier identification of organelles. *Comparative pathology* is again emphasized, and has been strengthened by using diseases that extend through different vertebrate species. Ontogeny and phylogeny are basic concerns in comparative medicine, particularly the tendency of closely related animal species to suffer similar metabolic, neoplastic, and infectious diseases. A section on *diagnostic electron microscopy* has been added for those who work day to day on disciplines that demand specialized technologies for electron microscopy. *Invertebrate pathology* is also included; although spontaneous diseases of invertebrate species are not mirrors of their counterparts in vertebrate animals, the biologic processes are similar and at the level of the cell may even be identical. One of the most exciting eras of pathology was begun by observations on the inflammatory response of the water flea. Lastly, *basic elements of description*—size, structure, and location in the cell—are emphasized throughout the book.

*Ultrastructural Pathology* contains electron micrographs acquired from my 40 years in the discipline at four different institutions. Over 75 micrographs have been contributed by electron microscopists working in comparative or medical pathology—all micrographs of exceptional quality.

To my many colleagues who have contributed to this book, I am deeply grateful. I acknowledge in particular the graduate students in veterinary pathology who have contributed ideas and micrographs (see dedication). The quality of electron micrographs is due in no small way to the technical staff of the National Animal Disease Center, particularly Judy Stasko, Karen Schlueter, Doris Buck, and James Heminover. Gene Hedberg has done outstanding work on many of the line drawings, and preparation of micrographs has been done by Wayne Romp and Tom Glasson. I am also grateful to the staff of Wiley-Blackwell, who have done a patient job of managing and editing the manuscript.
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Fig. P.1 Normal hepatocyte. Relationships of rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), peroxisomes (PO), mitochondria, and lipids in the cytoplasm, hepatocyte, dog. Cisternae of SER and RER communicate (arrows). Glycogen and lipid globules are associated with SER. Dense calcium-sequestering bodies are present in mitochondria.
In eukaryotic cells, control of function and development resides chiefly in the nucleus; metabolic and synthetic processes occur in the cytoplasm. The gel substance of the cytoplasm, the cytosol, is composed of a system of tiny filaments and various organelles and inclusions. Organelles are considered the internal functioning organs. Inclusions are sequestered accumulations of metabolites (lipid globules, glycogen, protein crystals, and pigments) that are not required to maintain cell life (Fig. P.1). To correctly describe or interpret a cellular structure in an electron micrograph, three important determinations are required:

- **Size**
- **Structure**
- **Location** in the cell

Injured cells develop changes in movement, density, and size. Cellular electrolytes are altered, metabolic pathways are interrupted or shifted, and structural changes develop in organelles. In the classic sequence of degeneration and death, the affected cells become pale and swollen, suffer irreversible injury from both direct and secondary effects, and collapse into shrunken, dark amorphous masses.

**Site of injury** is the critical factor in determining early cellular responses. Damage may be directed to cell surfaces, to mechanisms of nuclear control, to sites of energy formation in mitochondria, or to production of organelles in the cytoplasm. Nearly all injurious substances cause damage at multiple sites in the cells, and artificial emphasis on early dominant sites of injury must not obscure the complexity of cell degeneration.

Ultrastructural interpretation of pathologic change requires a systematic analysis of all components of the cell. Changes must be compared with the normal cell, including differences in location of the cell within the tissue and alterations that the normal cell undergoes in the process of diurnal or cyclical variation. For example, the normal hepatocyte is controlled and modified by hormones, cytokines, and stimuli of feeding and changes structurally during different stages of function.

Pathologic changes that develop in response to injury also depend upon (1) **duration of effect** and **concentration in tissue** of the injurious agent, (2) **metabolic characteristics of the cell**, and (3) **tissue vascular supply** and blood flow to the cell, including the amount of oxygen, the pH, and the temperature of circulating blood. Metabolically active cells are, in general, most susceptible to injury. Factors that increase metabolism predispose cells to injury. In liver, increased circulating thyroid hormones or increased dietary protein increase metabolism and oxygen consumption and augment cellular degeneration.

Injured tissues are typically composed of cells in various stages of degeneration, necrosis, or recovery. On one hand, injury may be so lethal that some cells are killed outright and appear within minutes as necrotic masses; conversely, other cells, because of their protected location or inactive metabolic state, appear to escape or to suffer only slight damage. Their manifestations of injury are not swelling and collapse, but a more slowly developing accumulation of molecules that the injured cell cannot process.

**Pitfalls** in the correct interpretation of electron micrographs are a lack of knowledge of conditions under which tissue was processed, especially the method of fixation. Osmium fixatives cause leaching of chromatin and other nuclear proteins, while glutaraldehyde is harsh on membranes. Different buffers and temperatures will also affect how cells appear.
Mitochondria are extremely labile, and even with careful fixation techniques, artifacts are common. Often there is artifactual change, accentuated because of pathologic swelling of the mitochondrion. All of these pitfalls are superimposed upon the dietary and environmental influences that affect the living animal.

Descriptions of structure must be clear and free of equivocal terms, especially inexact labels such as “fuzzy” or “almond-shaped.” Size, too often ignored, is the foundation of accuracy. The ribosome is approximately 22 nm in diameter and, since it is in nearly every cell, can be used for crude comparisons with unknown structures.

Because the sample size in electron microscopy is small, the most valid ultrastructural studies depend on quantitative or morphometric analyses to give a clear picture of change. The number and volume of organelles in cells from different mammalian species are relatively uniform. For example, livers of dogs and rats have similar amounts of endoplasmic reticulum, although dogs have slightly smoother and rats rougher endoplasmic reticulum. Dogs have double the volume of peroxisomes as rats, and rats have nearly twice as much neutral fat. Both are subject to cyclical nutritional variances.

In examination of any organ, one must systematically analyze blood and lymph vessels, nerves, and interstitial tissue that surround sites of injury. This includes mast cells, fibroblasts, and other cells. The vascular system should not be excluded from the descriptive process. Acute swelling in endothelial cells impedes blood flow mechanically and by binding surfaces of blood cells as they pass along injured endothelium. In either case there will be secondary effects on parenchymal cell populations.

Precise and accurate quantification of cellular changes in electron micrographs has traditionally used stereological tools to measure numbers of organelles as well as the surfaces, lengths, and volumes. Immunoelectron microscopy using gold labels in intracellular compartments expanded the use of morphology in cellular pathology, and quantitative analysis of gold labels in intracellular compartments added precision (see Mayhew, Histochem Cell Biol 119:332, 2003; Lucocq, J Hist Cyt 52:991, 2004).

The introduction of cryoelectron microscopy in the early 1980s allowed improved images and extension of resolution for determining viral structure from electron micrographs. In electron cryomicroscopy, unfixed sections are frozen in a thin (100 nm) layer of ice and a series of micrographs are taken at different angles. Fourier analysis is used to produce a three-dimensional (3-D) reconstruction.

The 3-D reconstruction begins with one or more electron micrographs in which each particle is a 2-D projection of a specimen. The relative orientation of each specimen, denoted by polar and azimuthal angle pair, defines the angle or view of the corresponding projection.

Electron microscopic tomography provides 3-D constructs of organelles (Figs. P.2–P.4). Individual tomograms are calculated from data on images obtained from samples on grids mounted on high tilt holders; final combined tomograms are viewed with specific software programs.

The coupling of stereology with electron tomography produces stacks of slices from electron microscopic sections—so-called quantitative 3-D electron microscopy. These parallel optical slices allow direct stereological analysis including number estimation with optical dissector methods and volume estimation based on the Cavalien principle.

Aberration-corrected electron lenses in transmission electron microscopy (TEM) allow structural studies at atomic-scale resolution. Use of aberration-corrected TEM determines the occupancies of atom sites and permits atomic-scale imaging of chemical composition and bonding (Uran, Science 321:506, 2008). This technology will be critical for explaining changes in nuclear structure and function in disease (Figs. P.5–P.7). Other new technology for ultrastructural analysis can be found on the following websites:

Microscopical Society of America: www.microscopy.org
Microbeam Analysis Society (instrumentation): www.microprobe.org

Whatever the method, one is cognizant that in vitro studies of ultrastructural change are artificial relative to changes in vivo and in ovo. Cellular organelles and pathways in vitro must alter to meet the demands of growth in the culture tube. For example, many of the alterations in cytoplasmic vacuoles based upon research in cell culture systems lack correlates for vacuolar changes of cells in the living animal. It is important that the microscopist does not become overly specific in identification of cytoplasmic vacuoles—specificity unjustified by the technique used for identification.
Fig. P.2  Tomogram of giant mitochondrion: lymphoblast, human Barth syndrome. A. Tomogram slice: surface-rendered depiction of a 3-D model, showing cristae in green and peripheral compartments (outer membrane + intermembrane space + inner membrane) in dark blue. Cristae are in honeycomb and concentric patterns. Boxes mark three segmented details, in which the outer membrane is bright blue and inner membrane is yellow. B–D. 3-D models of membranes in corresponding boxes: zones of inner membrane adhesion (white arrowheads), adhesions of inner membranes (red), and areas with open intracrista spaces (black arrowheads). (Micrographs: Devrin Acehan and Michael Schlame, Laboratory Investigation 87:40, 2007.)

Fig. P.3  Cryo electron microscopic tomography: Herpes simplex virus-1 entering a synaptosome. A. Capsid is inside the presynaptic element (left). Glycoprotein spikes are on the outer phase; tegument proteins correspond to local densities near the cytoplasmic area of the plasma membrane. B. Surface rendering of one virion and in synaptosome from the tomogram in A: capsid (light blue), tegument (orange), glycoproteins (yellow), cell membrane/viral membrane (dark blue), actin (dark red), vesicles (purple), synaptic vesicles (only partially segmented—metallic green), synaptic cleft (light green), and postsynaptic density (green). Scale bars, 100 nm. Micrographs: Ulrike E. Maurer, Beate Sodeik, and Kay Grünwald, Proc Nat Acad Sci USA 105:10559, 2008.)
Fig. P.4  Asbestos body: x-ray microanalysis spectra. A. Asbestos body TEM: characteristic annular morphology. Asbestos fragments are in the dark peripheral zone; bar = 2 μm. B. X-ray microanalysis map of asbestos body in A: localization of individual chemical elements in the coat of the body characterized by a silicon (Si) signal. Co-localization of iron (Fe) and chlorine (Cl); distinct distribution of iron (Fe) and calcium (Ca). Composition and localization of elements are shown (bottom). (Micrograph: H. K. Koerten, Am J Pathol 136:141, 1990.)
Fig. P.5  Nucleus: chromosome territories and nuclear bodies. (Drawing: R. Kumaran and David L. Spector, Cell 132:929, 2008.)

Fig. P.6  Nuclear envelope: inner nuclear membrane (INM) proteins, including lamina-associated polypeptides 1 and 2 (LAP1 and LAP2) and lamin B receptor (LBR), interact with HP1 and barrier-to-autointegration factor (BAF) and provide links to chromatin. Lamin filaments form the nuclear lamina. The outer nuclear membrane (ONM) cytoskeleton-associated nesprin proteins are tethered by Sun1 and Sun2 in the INM; the ONM is continuous with the ER. (Drawing: Colin L. Stewart, Brian Burke, Science 318:1408, 2007.)
Fig. P.7  **Nuclear activation**: nucleus of a secretory cell of the adrenal cortex sampled after marked anaphylaxis with associated massive stimulation for secretion corticosteroids. **Nucleolus** is large with a clearly defined nucleolus and much **perinucleolar chromatin**. Nuclear structures are (1) nucleolus, fibrillar part; (2) nucleolus, granular part; (3 and 4) interchromatin granule clusters (nuclear speckles).
Part I

Structural Basis of Cell Injury
Fig. 1.1  **Acute cell swelling**: hepatocyte, rat, *Pasteurella* type D toxin. There is increased cell volume, dilatation of the cisternae of the nuclear envelope and rough endoplasmic reticulum (RER), and dilatation of hepatic sinusoids (top right) and central vein. Note: endothelial cell degeneration in central vein. **B. Focal cytoplasmic vacuolation**: RER. **C. Vacuole margin**: dilatation of termini of RER sacculi (arrow) and vesiculation of membranes of the vacuole.
Acute Cell Swelling

Acute cell swelling is expansion of cell volume due to loss of control of water intake. It is a fundamental expression of acute injury, and is to the dying cell what electrolyte imbalance is to the dying animal, a basic killing mechanism over which are superimposed many other degenerative phenomena. With time, acute cell swelling progresses to a spectrum of changes that begin with clarification of the cytoplasm and extend to diffuse disintegration of intracellular proteins.

Dilatation of Cytocavitary Network Cisternae

The first evidence of acute cell swelling is dilatation in cisternae of the cytocavitary network: endoplasmic reticulum (ER), nuclear envelope, and Golgi complex (Fig. 1.1). Early changes are due to excess water uptake, which dilutes the cytoplasmic matrix and causes the cell to appear pale and relatively structureless. Membranes of cytoplasmic organelles pump ions between the cytosol and organelles to maintain water balance and, if injured, water accumulates in cisternae of the rough endoplasmic reticulum (RER). Cisternal spaces are wider than normal and the apposing membranes are less straight than in normal cells. With time, membranes of the RER become disoriented, so that the regular arrangement of saccules of the RER and Golgi complex become tortuous and bulbous. Diffuse dilatation of RER is one of the earliest signs of injury in most cells. If uncertain as to proper interpretation, examine the ends of the sheets of RER; it is here that water first accumulates.

Hydropic degeneration is a form of severe acute cell swelling characterized by water free in the cytosol and proteolysis arising from proteases activated by injury. In ultrastructural analysis, hydropic degeneration is used to indicate massive expansion of the cell by excess water and disappearance of proteins in the cytosol. In epithelium, hydropic degeneration is characterized by massive expansion of the cell and lysis of keratin fibrils. Referred to as ballooning degeneration, this pattern is common in vesicular disease.

Fragmentation of Cytocavitary Network Membranes

Rupture and fragmentation of membranes are invariable in acute cell swelling. As membranes of the endoplasmic reticulum and Golgi complex begin to fragment, water may accumulate in vesicles, cytoplasmic lakes, or vacuoles (Fig. 1.2).

Cytoplasmic Vesiculation

As membranes of the ER begin to fragment, many fragments reorient and reseal to form small vesicles. Continued function of membrane pumps causes water to accumulate in and to expand the vesicle. Cytoplasmic vesiculation is one hallmark of early acute swelling. Vesicles are formed from fragments of both RER and Golgi complex, and slightly later in smooth endoplasmic reticulum (SER). As swelling progresses, large numbers of vesicles replace areas normally occupied by components of the ER.

Cytoplasmic Lakes

Cytoplasmic lakes develop as gels of protein and water that are free in the cytoplasm. Histologically,
Fig. 1.2 Vacuolation: hepatocyte, rat, acute cell swelling of carbon tetrachlorine (CCl₄) toxicity. A. Hydropic degeneration (vesicular): cisternae of the cytocavitary network (endoplasmic reticulum, Golgi complex, and nuclear envelope) are distended with water. Note: stretched nuclear pores, lipid globules, and edema of pericellular space. B. Hydropic degeneration (vacuolar): vacuoles are distended with water, there is marked fragmentation of the cytocavitary network. Remnants of cytoplasm persist only around the nucleus and areas adjacent to the plasma membrane. The nucleus is small and has moved to an eccentric position in the cell. C. Cytoplasm at high magnification: note vesiculation and membrane fragmentation of rough endoplasmic reticulum (RER), ribosomal degranulation from RER membranes with accumulation of ribosomal fragments in the cytosol, and membrane vesiculation (vesical formation) of RER membranes that form large vacuoles.
lakes appear as eosinophilic inclusions; ultrastructurally they are opaque, non-membrane bound foci of delicate fibrils (Figs. 1.1B and 1.1C). Lakes are bordered by intact and fragmenting RER.

Cytoplasmic Vacuoles

Cytoplasmic vacuoles are filled with ions and water. Perisinusoidal vacuolation of hepatocytes is common in toxic disease, and in systemic diseases such as burns and heatstroke. Diffuse vacuolar degeneration is most common in parenchymal cells of liver, kidney, and other organs whose parenchymal cells bear large amounts of internal membranes that actively pump ions. Water moves rapidly into and distorts the endoplasmic reticulum and Golgi complex. In severe peracute injury, there is massive accumulation of water in large, and often coalesced vacuoles. This severe injury leads to lysis of the cell with liberation of cell fluids and proteins into tissue spaces. In liver, focal vacuolation develops in sublethal toxicities, typically first seen along sinusoidal margins of centrilobular hepatocytes, appears to originate by endocytosis at cell surfaces, and vacuoles often contain remnants of microvilli, the disintegration of which is also an early degenerative change.

Cytoplasmic Edema

Cytoplasmic edema, also called cytoplasmic rarefaction, is the hallmark of early cell swelling. As cell volume increases, the cytoplasm is diluted without an associated increase in cytoplasmic organelles. Cytoplasmic palor is seen as organelles and inclusions are separated by electron-lucent areas of cytoplasmic matrix, and there is the appearance of a loss of free ribosomes, endoplasmic reticulum, and glycogen. Tissue architecture is maintained, but the pale, enlarged cells press upon one another and normal tissue arrangements are distorted.

Cytoplasmic edema is most clearly evident in cells normally equipped to transport ions rapidly, for example, endothelium, renal tubular epithelium, and lining cells of the lungs, brain ventricles, and bladder. Acute cell swelling is especially significant in blood vessels, where endothelial cells are forced into the lumen and they impede blood flow by compressing intercellular junctions.

Ribosomal Degradation

Detachment of ribosomes from membranes of the RER results when protein synthesis is inhibited or redirected. In foci of ribosomal degranulation, detached ribosomes rapidly disintegrate, leaving the area filled with irregular ribosomal fragments that are smaller than the 22 nm diameter of normal ribosomes. With time, the detached ribosomes disappear, leaving a diffuse, finely granular background in the affected area. Ribosomal detachment is a manifestation of direct injury to ribosomal polymerases or to RER membranes, or occurs indirectly after primary injury to nucleolar structure on which ribosomes depend for their formation. Whatever the cause, detached ribosomes are blocked from their normal function of mRNA translation into peptide chains.

Cytoplasmic Proteolysis

As ribosomes disappear, proteases that degrade ribonucleoproteins in the cytoplasmic matrix are activated. As proteolysis progresses, the cytoplasm becomes cleared, at first in foci and then in large segments (Fig. 1.3). Acute swelling, in cells that contain large amounts of ribosomes or protein filaments, leads to cytoplasmic proteolysis, the progressive clearing and lysis of cytoplasmic proteins. Lysis of membranes and filaments leaves in its wake only an opaque background of protein debris. In skin and other keratinized epithelia, keratin fibrils are lysed, leaving a massive fluid-filled, turgid cell with only delicate fibrils remaining where solid keratin fibrils should be. Neuronal chromatolysis is lysis of rough RER that accompanies cell swelling and leads to large, pale neurons devoid of Nissl substance. Neuronal chromatolysis (not to be confused with chromatolysis related to disintegration of nuclear chromatin) is characteristic of injury caused by neurotropic viruses and of traumatic injury.

Peptide Fragment Accumulation

In severe acute cell swelling, protein synthesis is stopped. If the various phases of transcription, translation, and post-translational modification are not affected uniformly, excess peptides will accumulate and polymerize as aberrant fibrils or granules. These proteins may be free in the cytoplasmic matrix or nuclear matrix, or develop within cisternae of the cytocavitary network. Cells that secrete continuously, such as hepatocytes, commonly have combinations of fluids and proteins in secretory vacuoles. Water that accumulates in vesicles and vacuoles is mixed with secretory proteins and glycoproteins that are released into the fluids of the vacuole. Enzymes that cleave large macromolecules into active fragments (such as proinsulin to insulin) may act at the wrong site to cause the accumulation of abnormal cleavage products. Instead of being directed to the proper secretory granule, the abnormal peptides are shunted into the lysosomal pathway.
Fig. 1.3  **Acute cell swelling**. Hepatocyte, rat, CCl₄ toxicity.  
**A. General view:** vacuolation (V), accumulation of smooth endoplasmic reticulum, swelling of mitochondria (arrow), areas of degranulation and breakage of membranes of rough endoplasmic reticulum with accumulation of ribosomes. Capillary endothelium (C) is swollen, and there is debris in intercellular spaces.  
**B. Peroxisomes:** note association with damaged membranes.  
**C. Dilatation of nuclear envelope:** acute cell swelling. Nuclear pores are stretched and distorted.
Golgi Complex Vesiculation

**Golgi complex** injury causes formation of abnormal Golgi vesicles and retrograde changes in rough endoplasmic reticulum. **Golgi vacuolation** is invariable in acute swelling. Fragmentation and vesiculation of Golgi membranes contribute to the cytoplasmic vesicles of early cell swelling. The products of inhibited protein synthesis may accumulate in cisternae of the Golgi complex. Glycosylation of proteins and lipids is impaired in injured cells and abnormal peptides and lipoproteins accumulate within the Golgi vesicles.⁷⁸

**Intracisternal granules** of protein and lipids in Golgi saccules are common findings in diseases involving metabolic dysfunction. In hepatocytes and renal tubule cells, large spherical membrane-bounded inclusion bodies form within cisternae of the endoplasmic reticulum in Golgi vacuoles that develop in acute cell swelling. Within these structures, abnormal proteins (as well as normal cellular proteins) may polymerize into fibrils that interact to form crystalline lattices, a development that is enhanced by delays in fixation.

Glycogen-Lipid Droplet Ratio Reversal

Aerobic metabolism is suppressed in acute swelling and anaerobic glycolysis is activated to produce energy. There is a rapid **disappearance of glycogen**: simultaneously there is an **accumulation of lipid globules** in the cytoplasm. Neutral lipid accumulation results from suppressed protein synthesis, which blocks utilization of lipids for lipid-protein conjugation. Initially, the excess neutral lipids accumulate in small lipid globules (microglobular fatty degeneration) but in advanced degeneration the hepatocyte is distended with large globules of lipid (macroglobular fatty degeneration). Marked fatty degeneration, manifest as a massive increase of lipid globules, is most often a sign of subacute sublethal injury; rather than of severe acute cell swelling that leads to lysis. Most lipid globules develop in the cytoplasmic matrix (free, not membrane bound) adjacent to smooth endoplasmic reticulum. In severe peroxidative injury such as CCl₄ toxicity, lipid globules also form within the cytoplasmic network; the site depends largely upon the type of toxin involved.⁹

Smooth Endoplasmic Reticulum Proliferation and Aggregation

In sublethal toxic injury, the SER persists and proliferates; this is the structural manifestation of detoxification. The first evidence of SER alteration occurs discretely, in parts of the organelle located between mitochondria, and, in time, these areas develop large aggregates of SER membranes. Duplication of the SER is the expected change in the early stages of acute poisoning, which develops while other organelles degenerate in the early stages of acute cell swelling. In injury that develops within the SER, membranes fragment and form vesicles similar to those in injured RER.

**Calcisomes** are calcium pumping vesicles (similar to SER) that regulate intracellular Ca²⁺ flow in non-muscle cells. Calcisomes remain functional even in cells with severe swelling. In neurons, for example, disintegration of rough endoplasmic reticulum involves loss of most cytoplasmic structures, yet the calcisomes, those parts of the endoplasmic reticulum bearing calcium pumps, remain intact.

Mitochondrial Swelling

Mitochondria behave as osmometers, and the swelling that develops after injury reflects entry of solutes and water into the mitochondrial matrix. In severe swelling, excess water uptake leads to large, opaque mitochondria with broken cristae (Fig. 1.4). When primary injury to mitochondria is the cause of swelling, these organelles may be massively enlarged. Some toxins accumulate in or damage mitochondria selectively; for example, toxins that interfere with oxidative phosphorylation or electron transport in mitochondrial cristae will rapidly lead to ATP depletion and swelling. In lethal cell injury, the shut-down of ATP production is manifest as

- **Mitochondrial swelling**: water uptake from cytosol
- **Cristolysis**: breakup of cristae
- **Matrix lysis**: degradation of proteins in the matrix
- **Calcium-sequestering granule formation** in the matrix
- **Ballooned cristae**: water uptake plus electrolyte imbalance

Mitochondria take up Ca²⁺ from the cytosol and store it as hydroxyapatite when its concentration rises above normal; the rising cytoplasmic Ca²⁺ in degeneration typically causes an increase in size and number of calcium-sequestering granules. In lethal toxicity the Ca²⁺ imbalance may be so severe that foci of mineral develop within mitochondria to form crystalline arrangements.

Mitochondria are exquisitely sensitive to changes in many factors that control metabolism, for example, oxygen tension, water and electrolyte balance, pH, temperature, and glycolytic products that feed into the mitochondrial pathways. Degeneration thus occurs