Ultrastructural Pathology

SECOND EDITION

The Comparative Cellular Basis of Disease

Norman F. Cheville

WILEY-BLACKWELL

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Dedication

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:

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Gian Winkler

Contents

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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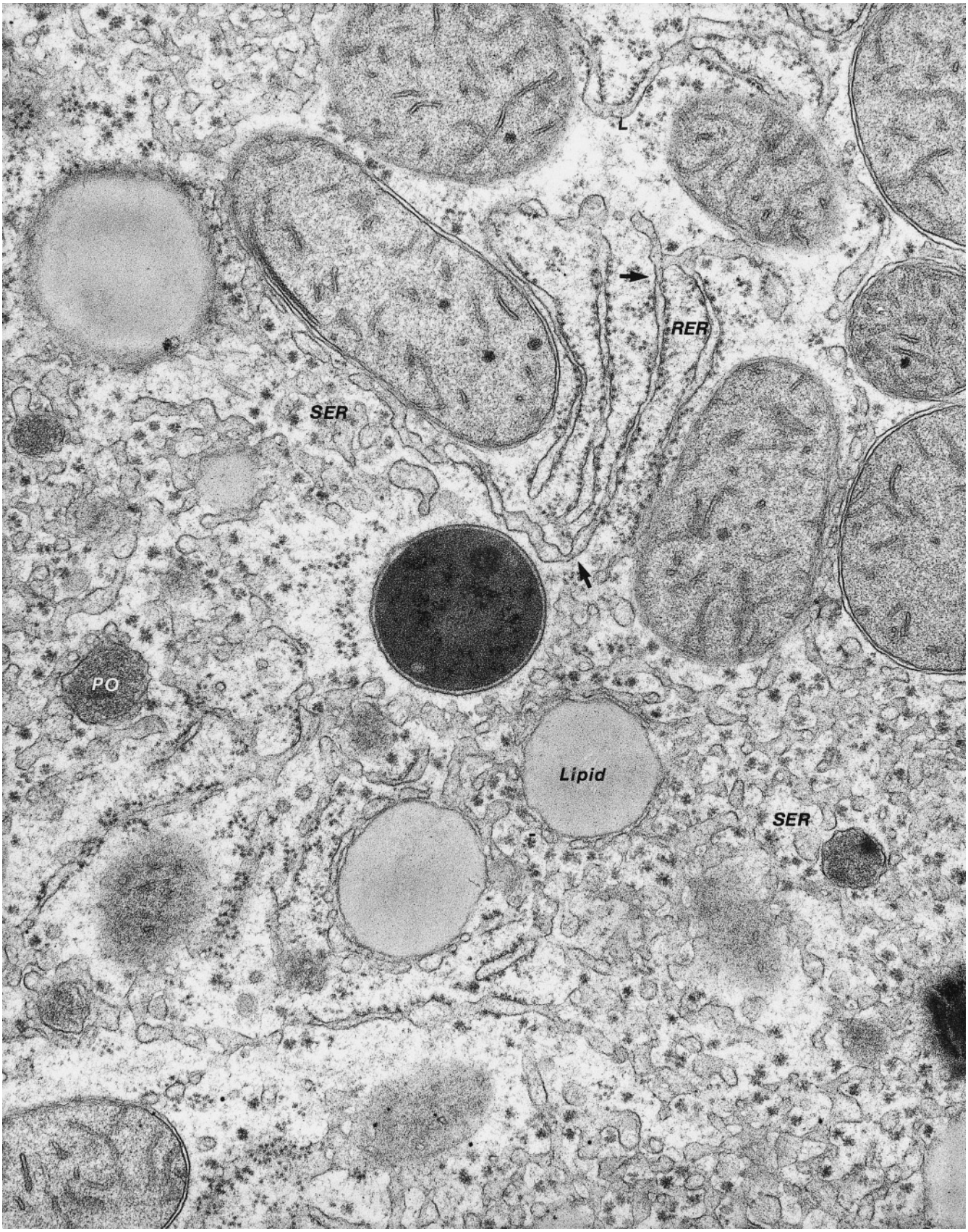

Fig. P.1 Normal hepatocyte. Relationships of rough endoplasmic reticulum (*RER*), smooth endoplasmic reticulum (*SER*), peroxisomes (*PO*), mitochondria, and lipids in the cytoplasm, hepatocyte, dog. Cisternae of SER and RER communicate (*arrows*). Glycogen and lipid globules are associated with SER. Dense calcium-sequestering bodies are present in mitochondria.

Prologue

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.

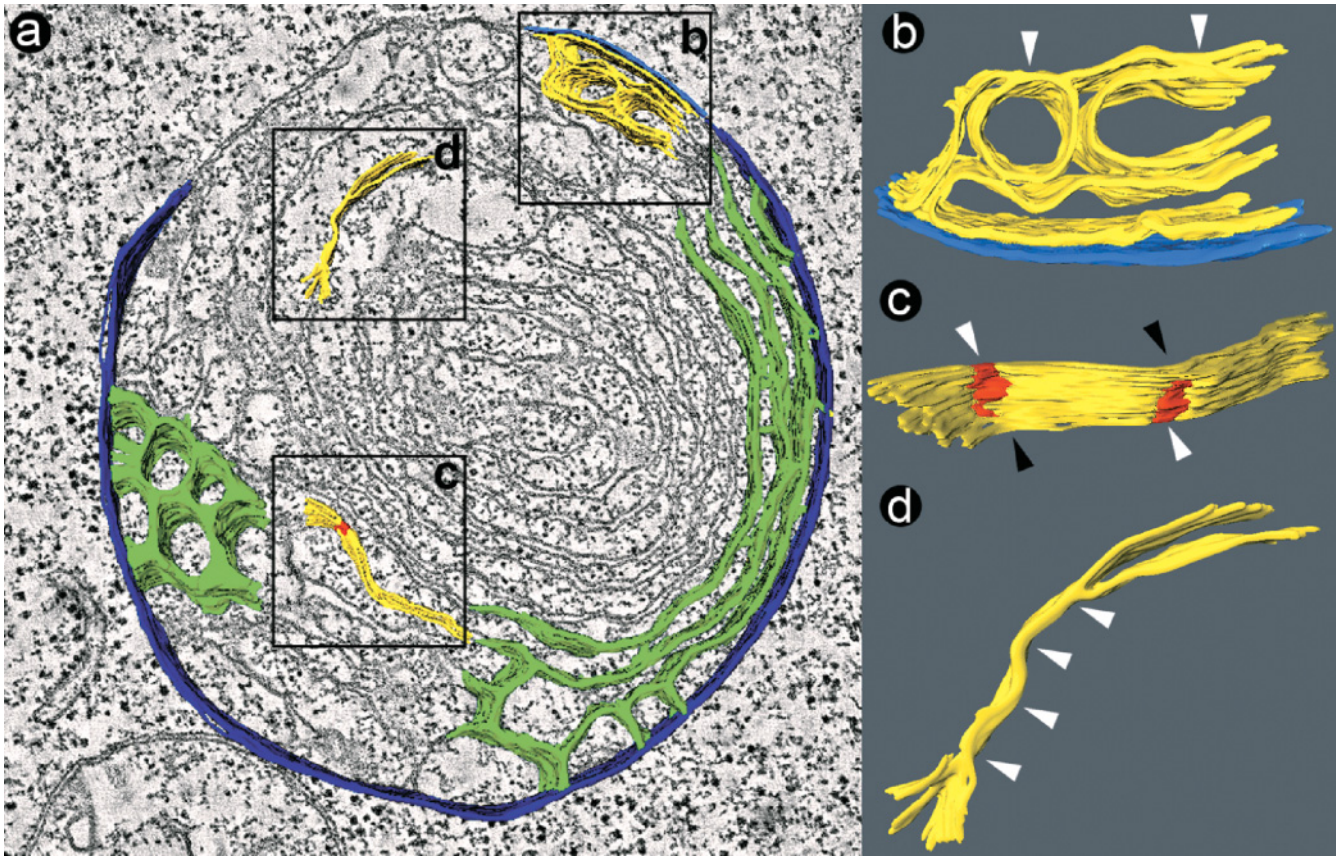

Fig. P.2 Tomogram of giant mitochondrion: lymphoblast, human Barth syndrome. **A**. **Tomogram slice**: surface-rendered depiction of a 3-D model, showing cristae in green and peripheral compartments (outer membrane + intermembrane space + inner membrane) in dark blue. Cristae are in honeycomb and concentric 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.)

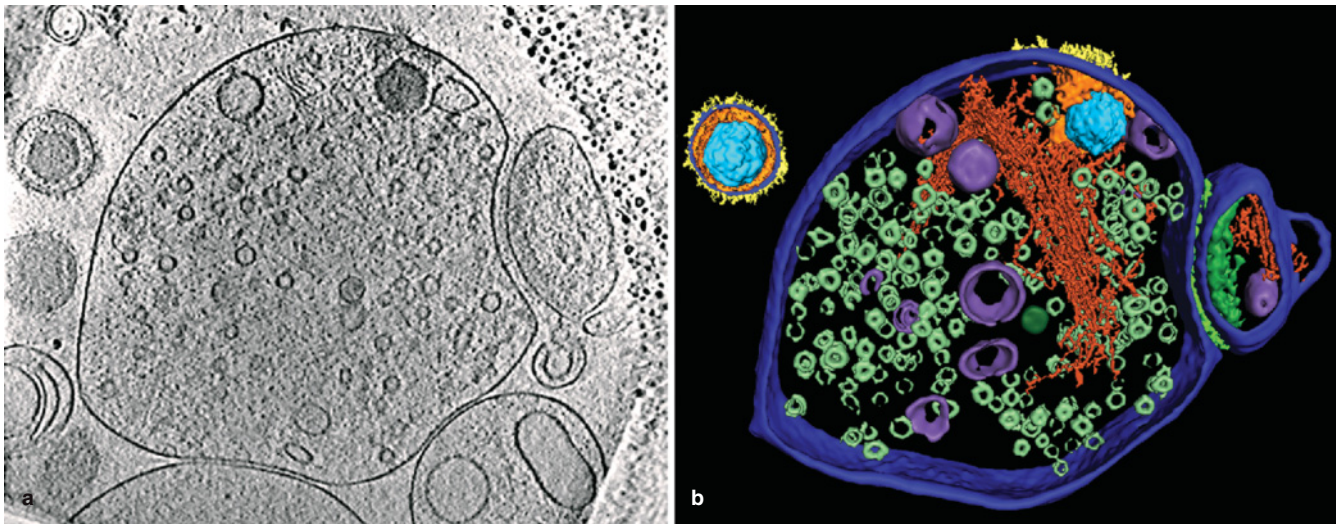

Fig. P.3 Cryo electron microscopic tomography: Herpes simplex virus-1 entering a synaptosome. **A. Capsid is inside the presynaptic element** (left). Glycoprotein spikes are on the outer phase; tegument proteins correspond to local densities near the cytoplasmic area of the plasma membrane. **B. Surface 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 Gruunwald, *Proc Nat Acad Sci USA* 105:10559, 2008).

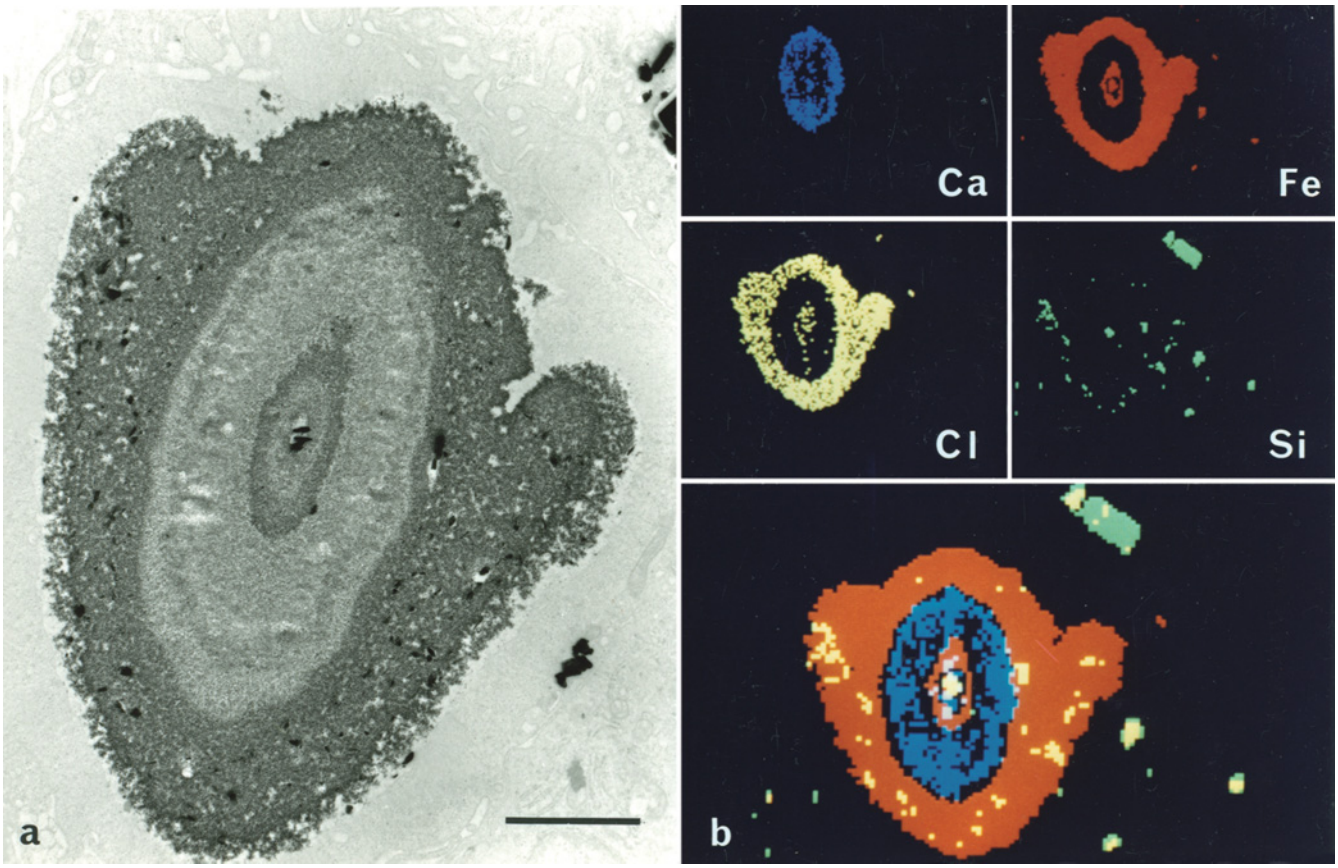

Fig. P.4 Asbestos body: x-ray microanalysis spectra. **A**. **Asbestos body TEM**: characteristic annular morphology. Asbestos fragments are in the dark peripheral zone; bar = 2 µm. **B**. **X-ray microanalysis map of asbestos body in *A***: localization of individual chemical elements in the coat of 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.)

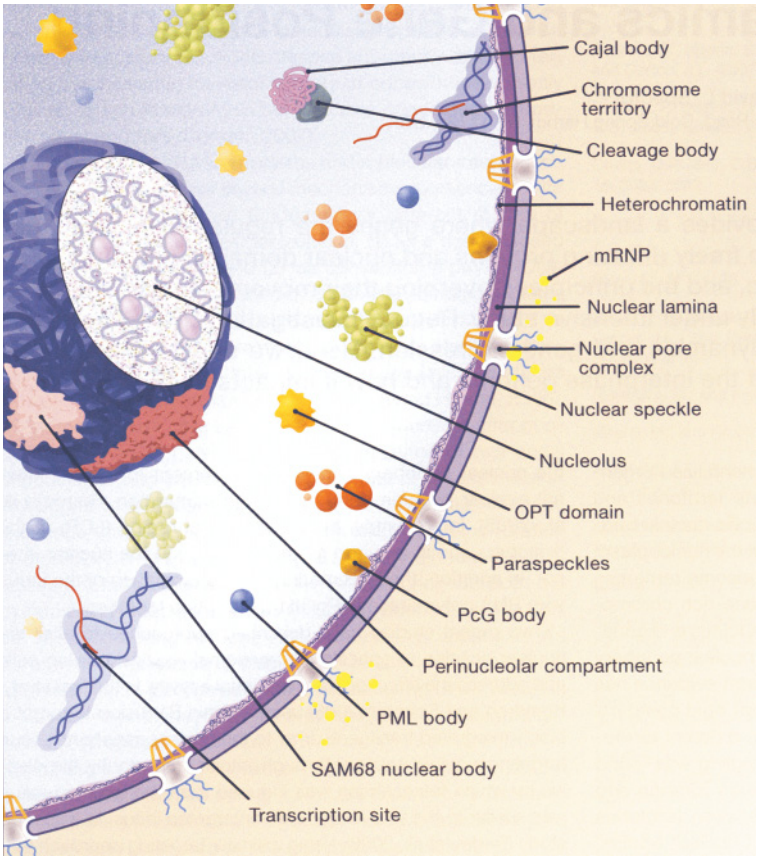

Fig. P.5 Nucleus: chromosome territories and nuclear bodies. (Drawing: R. Kumaran and David L. Spector, *Cell* 132:929, 2008.)

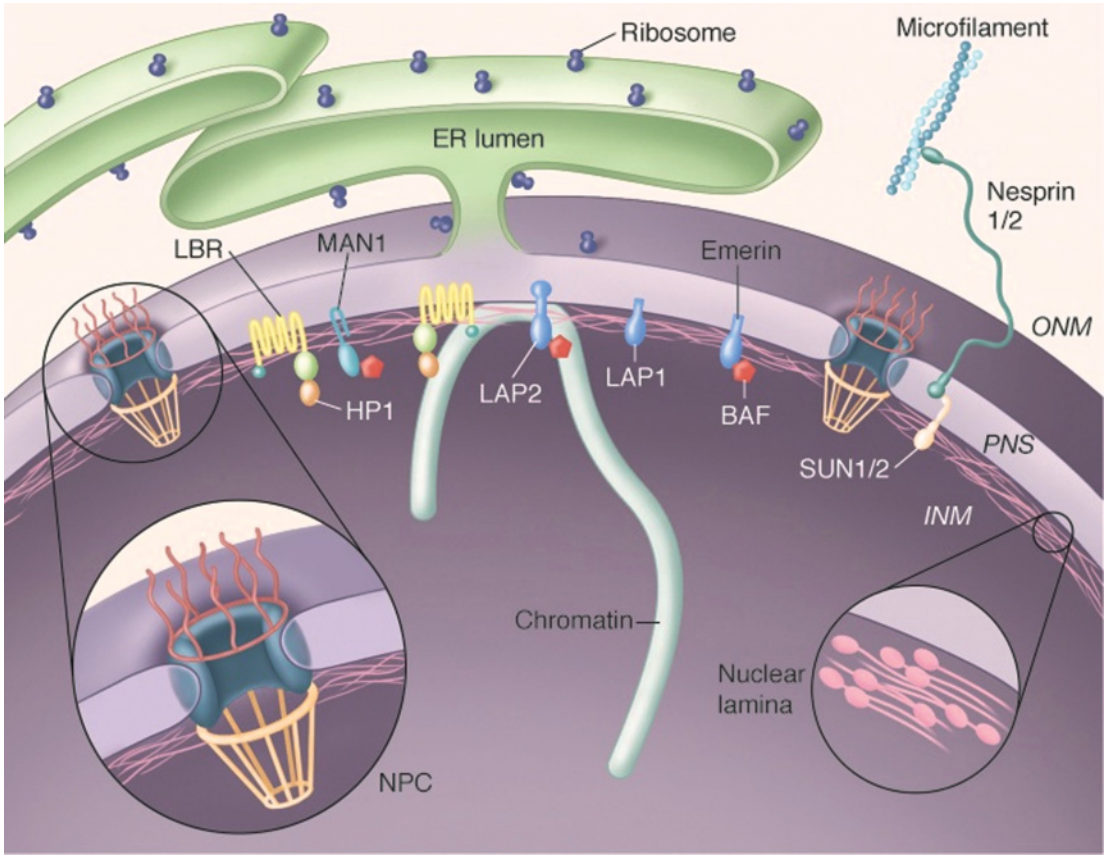

Fig. P.6 Nuclear envelope: inner nuclear membrane (*INM*) proteins, including lamina-associated polypeptides 1 and 2 (*LAP1 and LAP2*) and lamin B receptor (*LBR*), interact with HP1 and barrier-to-autointegration factor (*BAF*) and provide links to chromatin. Lamin filaments form the nuclear lamina. 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.)

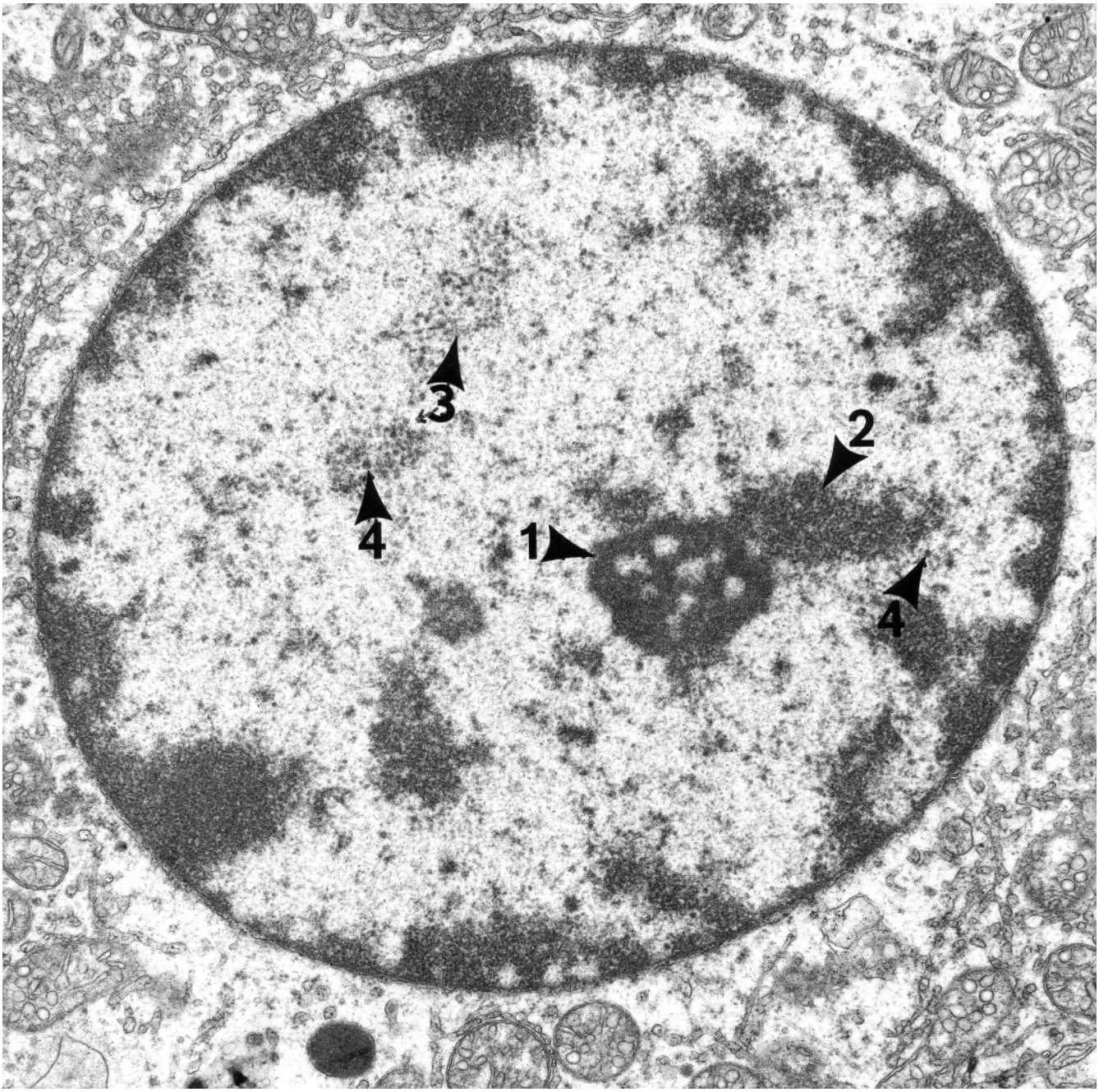

Fig. P.7 **Nuclear activation**: nucleus of a secretory cell of the adrenal cortex sampled after marked anaphylaxis with associated massive stimulation for secretion corticosteroids. **Nucleolus** is large with a clearly defined nucleolus and much **perinucleolar chromatin**. Nuclear structures are (*1*) nucleolus, fibrillar part; (*2*) nucleolus, granular part; (*3* and *4*) interchromatin granule clusters (nuclear speckles).

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Part I

Structural Basis of Cell Injury

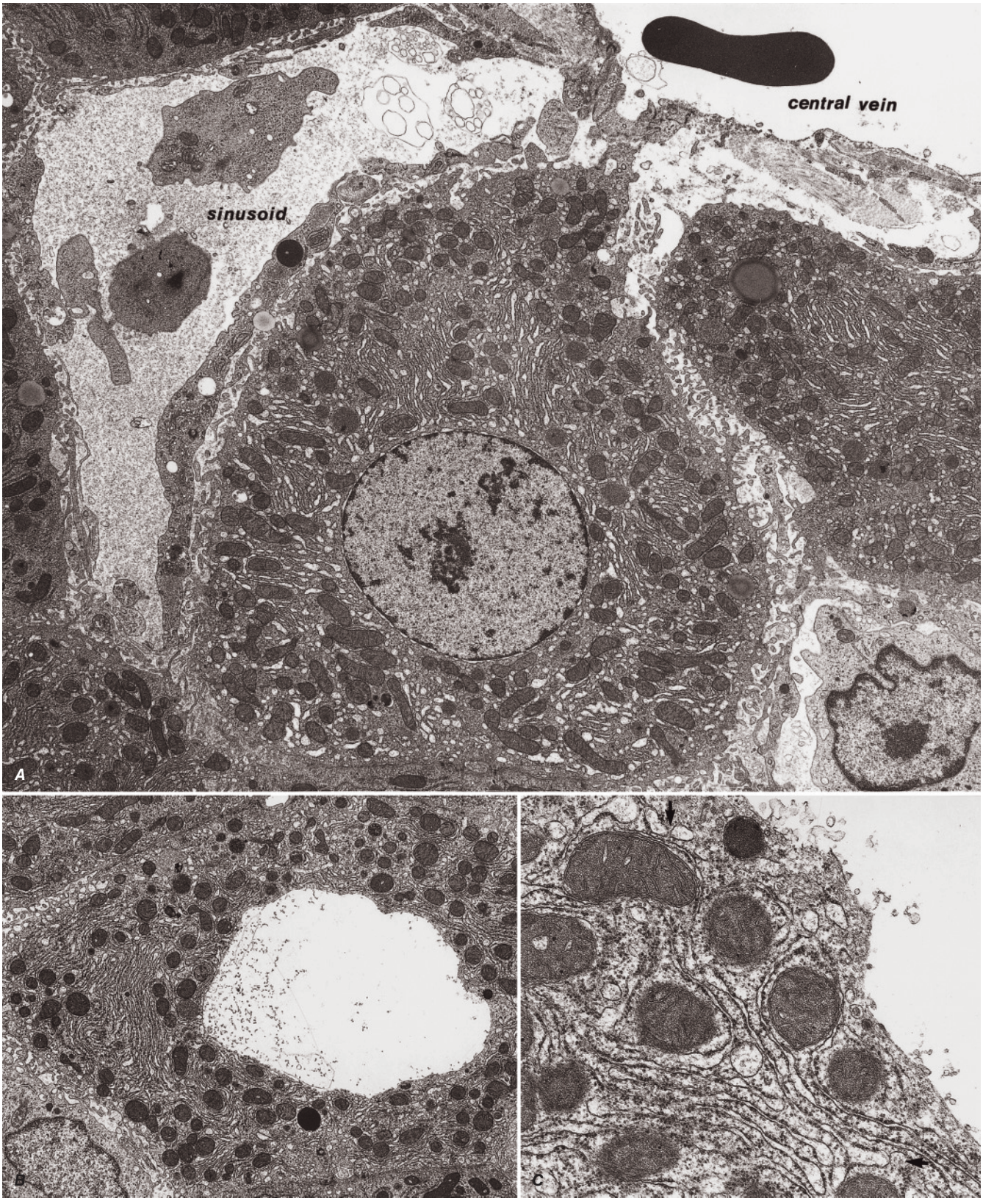

Fig. 1.1 **Acute cell swelling**: hepatocyte, rat, *Pasteurella* type D toxin. There is increased cell volume, dilatation of the cisternae of the nuclear envelope and rough endoplasmic reticulum (RER), and dilatation of hepatic sinusoids (*top right*) and central vein. Note: endothelial cell degeneration 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.

1 Response to Cellular Injury

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,

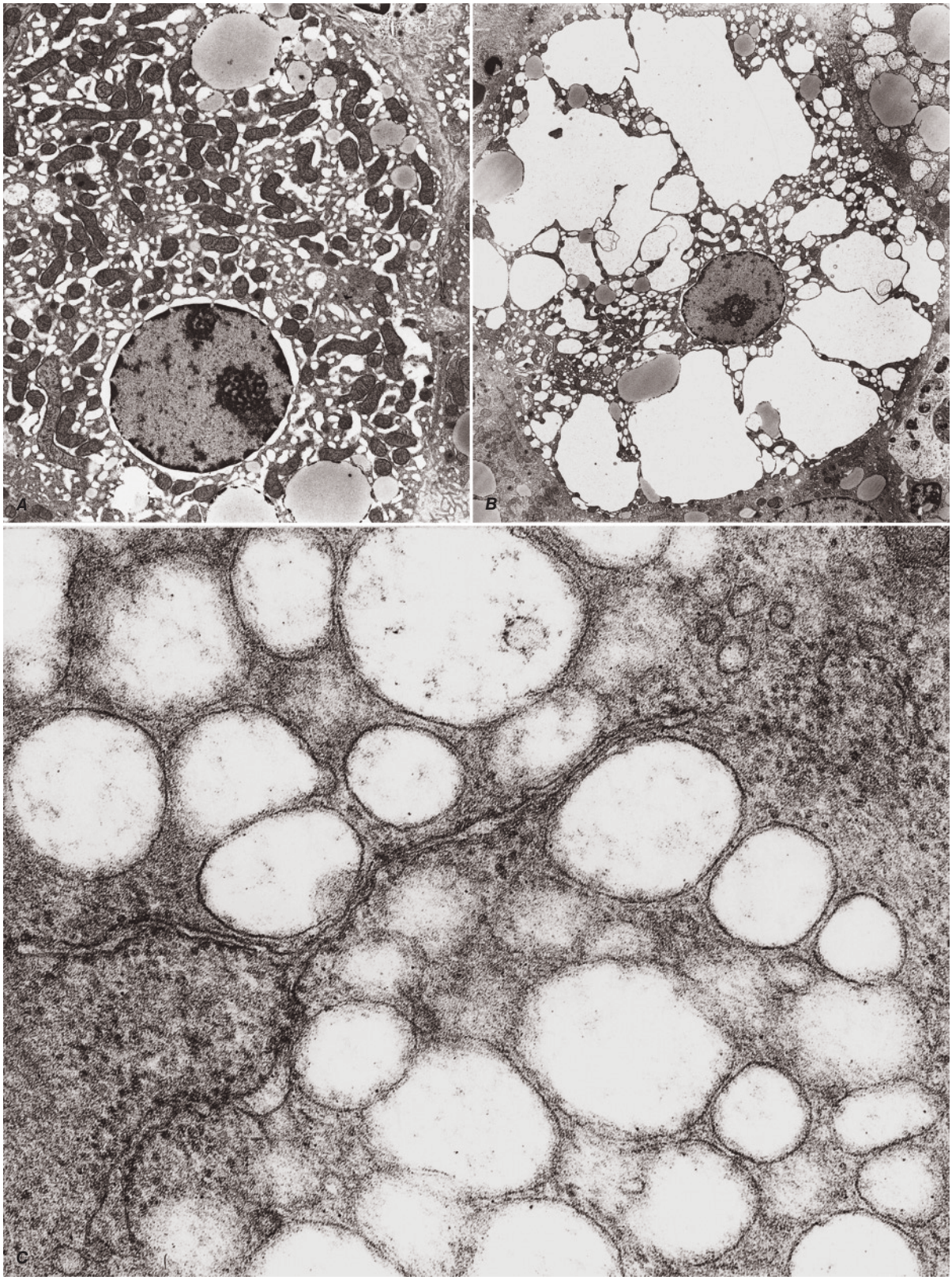

Fig. 1.2 **Vacuolation**: hepatocyte, rat, acute cell swelling of carbon tetrachlorine (CCl_4) toxicity. **A. Hydropic degeneration (vesicular)**: cisternae of the cytocavitary network (endoplasmic reticulum, Golgi complex, and nuclear envelope) are distended with water. Note: stretched nuclear pores, 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.[1] **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.[2] 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.[3]

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.[4–6] 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.

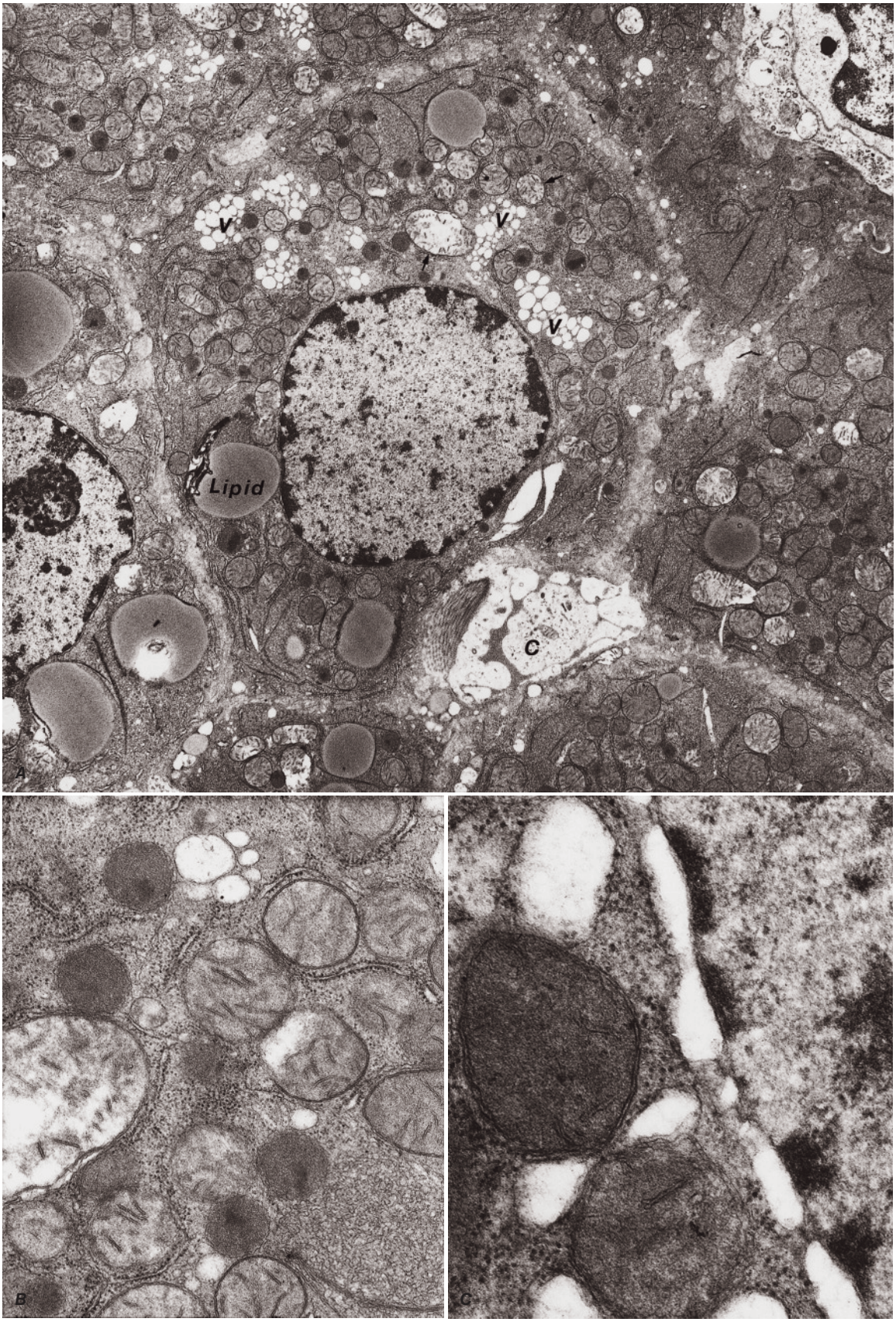

Fig. 1.3 **Acute cell swelling**, hepatocyte, rat, CCl_4 toxicity. **A**. **General view**: vacuolation (*V*), accumulation of smooth endoplasmic reticulum, swelling of mitochondria (*arrow*), areas of degranulation and breakage of membranes of rough endoplasmic reticulum with accumulation of ribosomes. 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.[7,8]

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 CCl_4 toxicity, lipid globules also form within the cytocavitary 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 Ca^{++} flow in nonmuscle 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 Ca^{++} from the cytosol and store it as hydroxyapatite when its concentration rises above normal; the rising cytoplasmic Ca^{++} in degeneration typically causes an increase in size and number of calcium-sequestering granules. In lethal toxicity the Ca^{++} 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

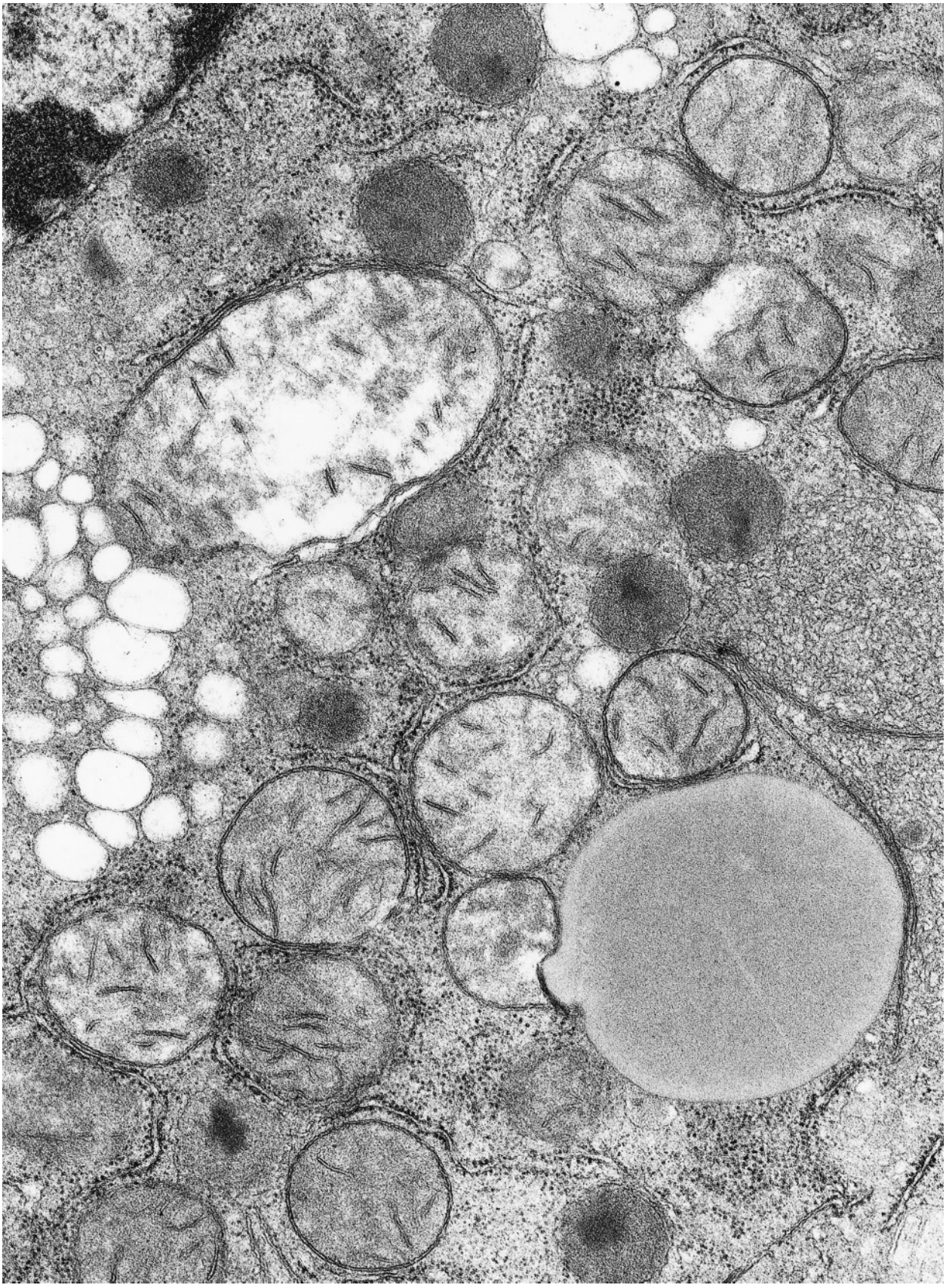

Fig. 1.4 Acute cell swelling, hepatocyte, rat, carbon tetrachloride toxicity.

secondary to almost every systemic insult to tissue and is widespread in conditions such a hypoxia, fever, acidosis, and toxemia.[10]

Mitochondrial Oxygen Deficit

Anoxia is rapidly translated into damage of mitochondrial cristae.[11] Oxygen is required at the end of the electron transport chain to accept electrons and to form water, and anoxia suppresses oxidative phosphorylation. The effects of abolishing oxidative phosphorylation are magnified by its tight coupling with the citric acid cycle. NADH accumulates and exerts a powerful effect to inhibit dehydrogenases of the cycle; for example, pyruvate dehydrogenase no longer will catalyze the flux of pyruvate to CoA. In acute **anoxia**, damage to mitochondrial membranes in electron micrographs suggests that shifts in ion balance are responsible for early damage (see the section in chapter 8 on oxidative stress).

Lysosome Activation and Autophagosome Formation

Autophagy is a general manifestation of injury. Sequestration of damaged organelles and abnormal proteins in **autophagosomes** is a mechanism of cellular repair and follows all types of sublethal injury. The fusion of autophagosomes with enzyme-bearing lysosomes forms the **autophagolysosomes** that degrade cellular debris. Internalization of damaged organelles such as cilia, microfilaments, microtubules, and cell junctions causes these structures to appear deep within the cytoplasm.

If cellular injury is severe, the cell may not be able to process debris adequately because of overload or exhaustion of lysosomal enzymes. Degradation of membranous cellular components may lead to accumulation of membranes that rearrange in **membranous whorls** or of proteins that aggregate into **lattice arrangements** within autophagosomes (Fig. 1.5). Cells then become swollen and foamy due to masses of large phagolysosomes or autophagolysosomes filled with inspissated granular debris, membranes, and lipids—a change sometimes referred to as **granulovacuolar degeneration** by light microscopists. Cells with high rates of membrane activity, such as steroid-producing cells of the adrenal cortex or interstitial cells of the testes, form large membranous whorls when they degenerate.

Membrane Fragment Accumulation

As these membranes fragment and degrade, membrane remnants accumulate as parallel strands or **concentric lamellar arrangements** that persist free in the cytoplasmic matrix. Membrane fragments are also taken into autophagic vacuoles, where they also form concentric lamellae. When the **endoplasmic reticulum** degenerates, many of the membranes collapse into long irregular fragments that aggregate and reorient into laminar arrangements. These membrane "bodies" are called **myelin figures** and are commonly found free in the cytosol. Myelin figures are especially frequent in cells with much endoplasmic reticulum; for example, in skeletal muscle cell injury, large myelin figures are found in the interfibrillar spaces. Membrane fragments are also taken into autophagosomes. Autophagosomes bearing myelin figures are referred to as **myeloid bodies**.

Secretory Granule Degradration

As secretory cells degenerate, secretory granules become pleomorphic and irregular, and are distributed in unusual sites in the cytoplasm. **Crinophagy**, the autophagic uptake of secretory granules, is increased as the cell attempts to rid itself of the damaged granules. In goblet cells, granular pneumocytes, and other cells bearing granules important in protection of injured surfaces, there may be a hyperplastic response with increased granule production and crinophagy.[12]

Tubulation

Tubular projections from organelles of the endoplasmic reticulum and the other components of the cytoplasmic vacuolar system (transport vesicles, endosomes, lysosomes, Golgi complex) develop as an early manifestation of acute cellular injury. Tubular projections extending from the membrane of Golgi saccules appear as long, thin 90 nm tubules. Tubulation is associated with damage to the coating process that guides the various forms of transport vesicles to the proper destination. As these coat proteins fail to function, large areas of the Golgi complex form these tubules, which move peripherally into the cytoplasm, dispersing enzymes of the Golgi backward into the endoplasmic reticulum.[13,14]

Increase in Peroxisomes

Peroxisomal duplication occurs in nearly all injuries to rapidly metabolizing cells. Pleomorphic peroxisomes are typically massed in groups at sites of cellular injury. If mitochondria are the primary site of damage, newly formed peroxisomes will accumulate adjacent to them, often with membranes of the two in close apposition. In liver, centrolobular hepatocytes

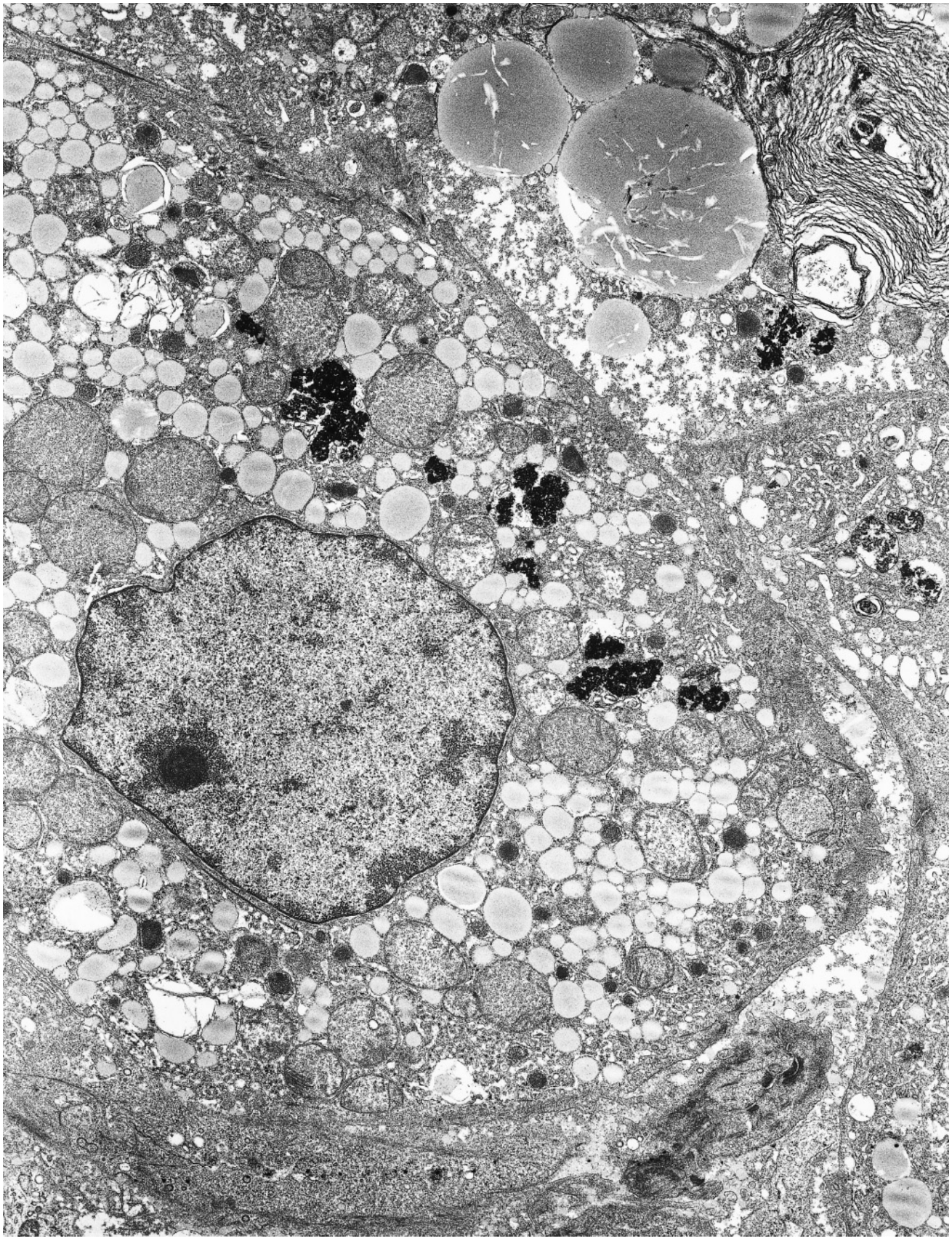

Fig. 1.5 Autophagy and steatosis: hepatocyte, dog, subacute toxic hepatic necrosis. Increased numbers of lipid globules reflect a marked production of triglycerides. Autophagic vacuoles are present throughout the cell. Note: myelin figures (*top right*), mitochondrial cristolysis, and accumulations of matrix proteins and debris in interstitial spaces.

are most sensitive to peroxisomal proliferation; abnormal peroxisomes with matrix tubules are apt to occur in more peripheral areas.

The Nucleus in Acute Cell Swelling

Nuclear Pores Disintegrate

The **nuclear envelope** undergoes major changes in acute cell swelling. It becomes turgid and the perinuclear space dilates. **Nuclear pores**, which are avenues for exchange of water, ions, and proteins between nucleus and cytoplasm, become stretched, lose their **septae** (diaphragms), and disintegrate. The network of delicate **fibrils** that connect adjacent pores and radiate inward from the pores into the nucleoplasm is affected early, especially those fibrils that connect adjacent pores. At the cytoplasmic face of the nuclear envelope, the cytoskeleton collapses around the nucleus. This collapse may reinforce the closure of nuclear pores that protect the nucleus from toxins in the cytoplasm.

Nucleoli Become Eccentric and Disintegrate

Nucleoli move to the periphery of the nucleus. **Nucleolar margination** is an attempt to facilitate nuclear-cytoplasmic interchange in early cell injury. As injury progresses however, this is ineffective and the nucleolus begins to degenerate. When severely injured, nucleoli disintegrate in the following progression:

- **Disassembly of fibrillar centers** and surrounding **dense fibrillar components**, a change caused by diminished transcription of rRNA genes by RNA polymerase I and leading to aggregation and disappearance of fibrillarin and small nuclear RNAs
- **Disassembly of the granular component**, which is brought about by the diminished assembly of preribosomal particles
- **Dissociation of components**, the fibrillar component, granular cortex, and intranucleolar chromatin
- **Segregation of fibrillar and granular components** into distinct zones
- **Disappearance of fibrillar and granular areas**

Nucleolar damage is most obvious when DNA or its polymerases are specifically injured.[15,16] For example, aminonucleoside antibiotics selectively inhibit the nucleolar enzyme RNA polymerase I and block production of preribosomal RNA, the product of this enzyme; affected nuclei segregate, form microspherules, and fragment, and the remaining nucleolar components condense.[17]

Chromatin and Nuclear Proteins Aggregate

Clumping of chromatin accompanies cell injury. Granules of chromatin disappear from the central karyoplasm as chromatin increases in areas adjacent to the inner side of the nuclear envelope. Attributed, in part, to the lowered pH of acute cell swelling, this change reverses upon recovery. **Perichromatin granules**, electron-dense solitary 30–35 nm granules with a surrounding halo, accumulate around nucleoli in some toxic reactions, a change associated with decreased protein synthesis.[18,19]

In sublethal injury, chromatin may be depleted or there can be massive deposits. In severe injury,

- **Small fragments of chromatin are randomly formed in the karyoplasm**: a change caused by indiscriminant breaking of chromatin strands (Fig. 1.6)
- **Chromatin segments are small and uniformly dispersed** throughout the karyoplasm: chromatin segments have been cleaved evenly
- **Massive crescent-shaped deposits** of chromatin adjacent to the nuclear envelope: a change caused by minimal breaks of single strands of DNA by specific endonucleases without further proteolytic breakdown (see apoptosis, chapter 2)

Excessive production of protein (or failure to degrade protein) leads to deposition of proteinaceous granules or filaments, most often from inappropriate aggregation of nucleoproteins. Massive aggregates of proteins or protein-membrane complexes appear as **intranuclear inclusions**, and are common in diseases caused by the DNA viruses, and by toxicity of heavy metals such as lead and bismuth. **Nuclear pseudoinclusions** (formed from invaginations of the nuclear envelope back into the nucleoplasm) must be differentiated from true nuclear aggregates.

DNA Damage

DNA damage is a common sequela of injury, particularly in neurons of the traumatized brain. Experimental studies in the traumatized rat brain reveal single- and double-strand DNA breaks and suggest that there are several pathways in the evolution of DNA damage after trauma.[20] Cytochemical techniques for in situ identification of DNA damage include DNA polymerase I-mediated biotin-dATP nick-translation (PANT, for single-strand DNA breaks), terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL, for double-strand DNA breaks), and the Klenow fragment of DNA polymerase I-mediated biotin-dATP nick-end labeling (for single and double breaks).

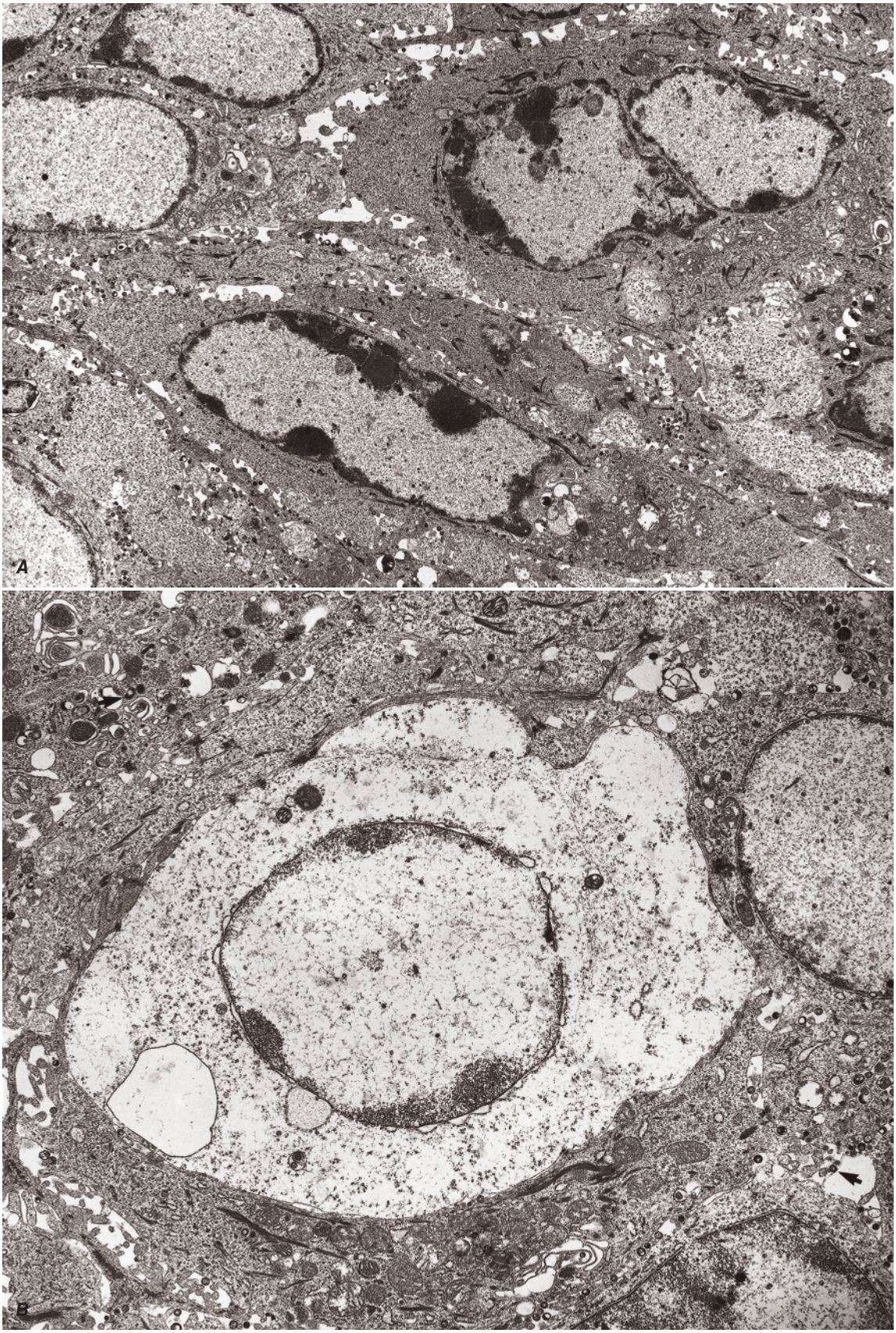

Fig. 1.6 Nuclear and cytoplasmic proteolysis: keratinocyte, cow, acute cell swelling, herpesvirus infection (infectious bovine rhinotracheitis). **A**. **Nuclear inclusions**: peripheralization of chromatin and nucleoli, and lysis of nuclear matrix. **B**. **Nuclear membrane breakdown**: there are lysis of cytoplasmic components, vacuoles and lysis of nucleoplasm with viral nucleocapsids in nucleoplasm and virions outside the cell (*arrow*). Note the disintegration of the nuclear envelope with liberation of nucleocapsids into the cytoplasm.

Rarefaction of Nucleoplasm

Nucleoplasmic rarefaction is a hallmark of acute swelling. After injury, enzymes are activated that degrade and lyse proteins and nucleic acids. Rarefaction of nucleoplasm is accompanied by clumping, or **peripheralization of chromatin** along the inner surface of the nuclear envelope. Chromatin clumping is most frequently a consequence of decreased cellular pH that occurs with accumulation of lactate. **Focal nucleoplasmic rarefaction** is uncommon but does occur in cellular injury that is slight and prolonged, typically where discrete metabolic pathways are specifically blocked.

Nuclear Envelope Collapse and Invagination

In severe injury, the nuclear surface becomes highly irregular and may invaginate on itself. Duplication of membranes of the envelope results in blebs that protrude into the cytoplasm. More commonly, the pressure of swelling causes invagination into the nucleus of focal areas of the cytoplasm, which, in cross section, appear as membrane-bound inclusions called **pseudoinclusions**. **Evagination** or **blebbing** of portions of the nucleus into the cytoplasm has been reported, but many of these types of inclusions are fixation artifacts. The outer membrane of the nuclear envelope does, however, bulge into cytoplasmic areas, usually in association with annulate lamellae formation.

Nucleus Contracts

Contraction of the nucleus with precipitation of chromatin into dense masses is the hallmark of lethal injury. Rupture of the nuclear membrane with fragmentation and release of nuclear contents, called **karyorrhexis**, terminates as **karyoklasis**. This total breakdown of the nucleus leaves only dense chromatin aggregates in the necrotic cell.

Plasma Membrane Is Irregular or Discontinuous

Structural defects in the **plasma membrane** and its **glycocalyx** and in the **cytoskeleton** below the membrane reflect a progressive loss of cellular architecture in acute swelling. Massive changes in the distribution of integrins, receptors, and adhesion molecules occur at the cell surface, and enhance the process of swelling and segregation of the cell from its neighbors.

Morphometeric studies of acute swelling clearly show a marked increase in cell volume. Swollen epithelial cells bulge from the normal limits of the epithelium. Extending outward into the surrounding cavity or lumen, the cell surface is irregular or bulbous, and by scanning electron microscopy can be seen to contain waves and pits. Epithelial surfaces develop a cobblestone appearance as open pits appear where cells have desquamated. Normal cells adjacent to swollen cells flatten and become dome-shaped as they move laterally to cover the basal area after the bulging degenerate cell is squeezed from the epithelium.[21]

As the cell surface is injured, integral proteins embedded in the plasma membrane move laterally in the membrane, away from the site of injury. Although not detectable by routine microscopy, this change in membrane protein distribution can be readily seen by freeze-fracture techniques. The protein knobs that are usually regularly distributed over the surface are missing at sites of injury. In some cases, lacerations of the membrane can be detected in these areas.[21]

Transmembrane proteins, which have random distributions in the plasma membrane, become aggregated or disappear.[22,23] By scanning electron microscopy, changes in membrane particles can be assessed and quantified. During acute cell swelling, integrins and other adhesion receptors are internalized by endocytosis, and this leads to disassociation, detachment, and exfoliation.[24]

Cell Surfaces Are Pitted

Surface pitting associated with accelerated endocytosis occurs in acute cell swelling of some cell types, especially in motile cells such as lymphocytes and erythrocytes. Surface pitting is a characteristic degenerative response, and scanning electron micrographs of cells injured by various membrane poisons reveal surface pits and craters. In experimental studies in vitro on cellular degeneration of thymocytes caused by corticosteroids, surface pitting was a result of exocytosis involving vesicles aggregated at the cell surface.[25]

Focal Lysis of Plasma Membrane

In discrete injury, focal discontinuities may be present in the lipid bilayers of the cell membrane (Fig. 1.7). This change should not be reported unless fixation of the specimen is rigidly controlled and accompanied by appropriate controls. Even then, it is best to have evidence that the agent involved is located at the site of injury. If a specific toxin, enzyme, or porin can be identified by immunolabels attached to the membrane, a discrete defect can be confirmed.

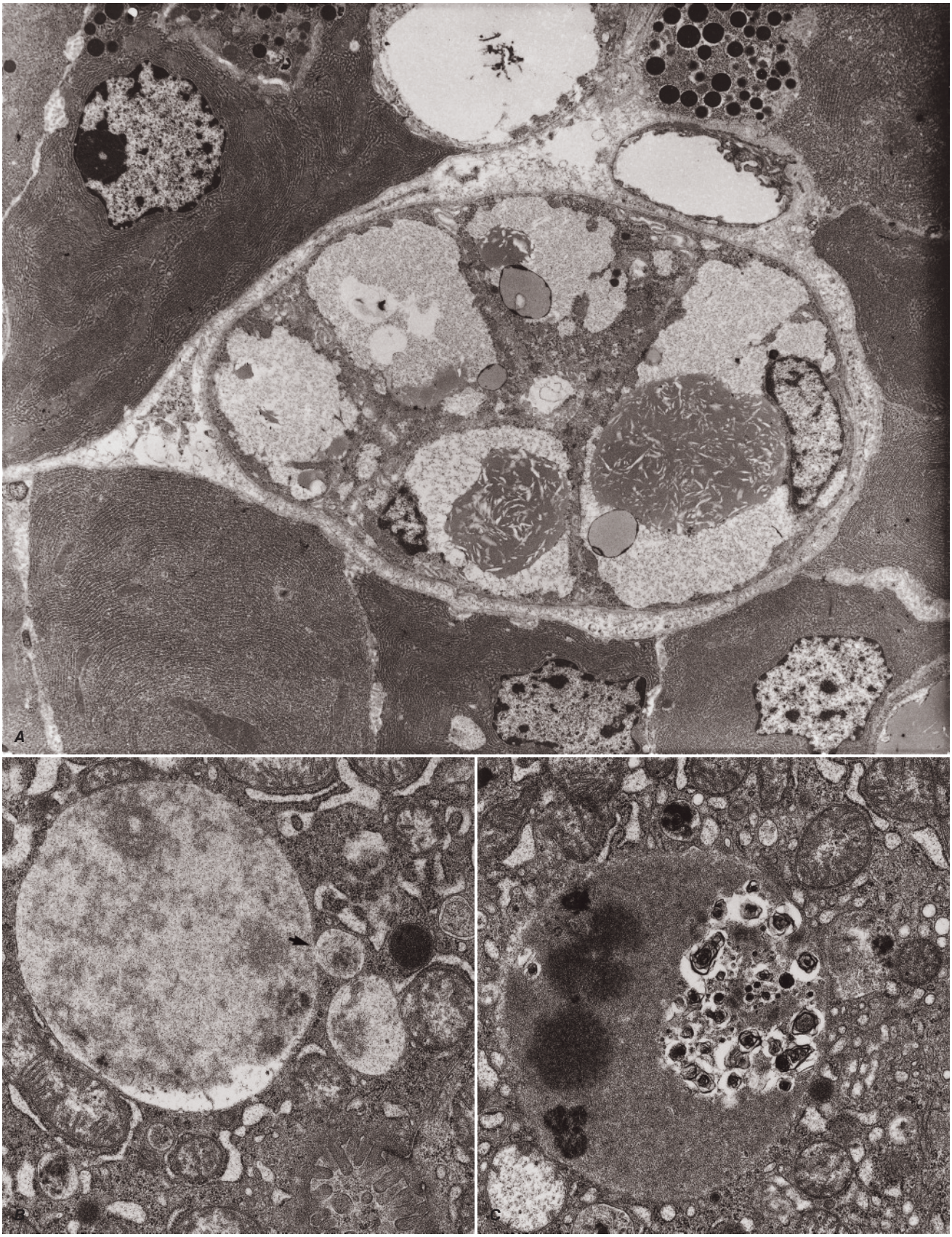

Fig. 1.7 Cytoplasmic lesions. **A**. **Acute cell swelling with glycogen and lipid**: pancreatic ductule, dog, acute pancreatic necrosis. Escape of enzymes into interstitial tissues causes necrosis in all components of tissue including capillary (*top right*), acinar cells, and ductal epithelium. **B–C**. **Proteinaceous vacuoles (hyalin globules)**, liver. **B**. **Early stage**: multiple granular inclusions formed by fusion of small protein-bearing vacuoles (*arrow*). **C**. **Late stage**: large dense proteinaceous inclusion bearing myelin figures and protein debris.

Granulovesicular Debris Accumulates in Intercellular Spaces

As acute swelling progresses, tiny granules of protein debris accumulate, and the cytoplasm becomes blurred and disorganized. Membranes, because they become undulant, irregular, and twisted, are less distinct. At cell surfaces, accumulation of debris in interstitial spaces masks alterations that have occurred in the plasma membrane, microvilli, and intercellular junctions, and makes the interpretation of change difficult. The filling of intercellular spaces with granular debris and vesicles is a hallmark of advanced acute cell swelling. In low-power electron micrographs these spaces are clear and cells are closely apposed in most normal tissues; in acute cell swelling the membranes are indistinct and spaces are electron dense because of the debris.

Cell-to-Cell Contact Is Disrupted

The normal close apposition of parenchymal or epithelial cells is disrupted as cells are injured and acute swelling progresses. Cell junctions disintegrate and cells lose their normal cohesiveness with neighboring cells. Production of adhesion molecules and counterreceptors is suppressed, and this leads to separation of cells from their neighbors and sloughing from the surface. Internalization of persisting adhesion molecules by endocytosis enhances cell-to-cell detachment. Sloughing of epithelium results from loss of cell-to-basal lamina contacts; that is, the connections formed by hemidesmosomes and adhesion plaques with the extracellular matrix and basement membrane. In human renal biopsies from patients with acute tubular necrosis, there is focal epithelial loss in the nephron. Some single cells or arrays of cells are detached from the underlying basement membrane but are held in place by a few remaining junctional attachments to neighboring cells.[26]

Gap Junctions Close

One of the first events to occur in acute swelling, the separation and shut-down of gap junctions, is brought about by decreasing pH (cellular acidosis) and increasing calcium concentrations.[27] Gap junctions function in intercellular communication to transmit small molecules (just under the size of glucose) from cell to cell. The closing of gap junctions inhibits the spread of injurious agents and lethal amounts of cytokines. Gap junction loss may be a subtle change in micrographs taken by standard transmission electron microscopy. When hepatocytes affected by acute swelling are examined by freeze-fracture techniques, **connexons** (the large protein complexes that make up the channels in gap junctions) are decreased. Connexon proteins separate, disintegrate, and are dispersed within the plasma membrane and, in severe injury, are removed by internalization and autophagy. Gap junctions rapidly become disordered in swollen ischemic cardiac myocytes in human myocardial infarcts. When examined by immunolabeling techniques, gap junctional proteins, instead of being discretely aggregated at the intercalated disk, are widely diffused over the cell surface.[28]

Tight Junctions Disassociate

As cytoplasmic ionic balances shift, the plasma membrane becomes leaky and fluid accumulates in the cell. Because capillary endothelium is also affected at sites of injury, fluid tends to leak into and accumulate in interstitial spaces, isolating cells from one another and accentuating cellular "individualization." As this process proceeds, **tight junctions** disintegrate and cells tend to remain attached only at desmosomes.

Hemidesmosomes Detach

Hemidesmosomes maintain the attachment of basal surfaces of epithelial cells to the underlying basement membrane. After injury, hemidesmosomes are irregular and frayed. With special techniques, the anchoring fibrils can be shown to be missing from the subbasal lamina lucida. In the process of acute cell swelling, hemidesmosomal proteins disintegrate and the link between epithelial cell and basement membrane is severed.

Desmosomes Disintegrate

Desmosomes, the intercellular junctions that connect adjacent cells by strong mechanical coupling, are remarkably resistant to injury. They are the last cell junctions to disintegrate in severe acute cell swelling. As swelling progresses, however, desmosomes disintegrate. The dense desmosomal components separate from the plasma membrane, evaginate into the cytoplasm, are endocytosed, and are transported to lysosomes for degradation. **Intermediate filaments**, which are anchored in the desmosomal plaque, are released and aggregate in the cytoplasm.[29]

Cytoskeleton Disassembles

Disassembly of components of the subsurface cytoskeleton may be a direct effect of toxins, or may be secondary to degenerative effect elsewhere in the cell. In either case, there is disintegration of **microfilaments**

(and the linker proteins that connect them to the plasma membrane), and of **intermediate filaments** that stabilize on intracellular junctions. This disruption of the peripheral cytoskeleton causes the cell to become rounded and to separate from its neighbors, a process referred to as **individualization**.

As a general response to acute cell swelling, microfilaments disintegrate into irregular globular aggregates of actin.[30] This change is difficult to assess in routine preparations but can be demonstrated by special immunolabeling techniques. Some toxins cause microfilaments to accumulate; actin polymerization is excessive and actin is assembled into large bundles of microfilaments. As with microfilament loss, the functional connections of actin with the plasma membrane and its surface structures are inefficient.

Other components of the cytoskeleton may be abnormal. **Intermediate filaments** thin or thicken with different types of injury.[31,32] Specific injury is directed to **microtubules** by colchicine[33,34] and by halothane and other volatile anesthetics, which cause **dissociation of microtubules** into component filaments with aberrant reassembly into twisted ribbons.[35,36]

When the cytoskeleton is damaged diffusely, cells rapidly change shape, becoming rounded. Loss of cortical structural support is associated with degradation of surface structures and shedding of massive areas of cytoplasm into interstitial areas, desquamation from lumenal surfaces, or embolization of large cell fragments.

Surface Structures Are Disorganized or Missing

Filopodia Are Altered

Injured cells typically have surfaces that are irregular and undulant, with **filopodia** of increased length and number. Filopodia are important in establishing connections with other cells, and are critical for the binding of macrophages and inflammatory leukocytes to each other, and to endothelial cells of blood vessels. Filopodia have binding properties that exceed other areas of the cell surface because their plasma membranes are rich in integrins and other adhesion molecules. Filopodia not only bind to other cells but also attach to bacteria and other microorganisms.[37]

Microvilli Are Distorted

With progressive acute cell swelling, the cell surface becomes disorganized and microvilli become irregular and are lost from the cell. Microvilli that remain are generally shorter than normal but, in some types of injury, as some microvilli disappear, others become longer. In acute toxic injury the actin core of the microvillus disintegrates, core microfilaments lose connections with the plasma membrane covering the microvillus, and the microfilament bundle disintegrates, causing the microvillus to shorten. In the kidney, microvilli of the proximal convoluted tubule are highly susceptible and rapidly disappear after injury. This is also characteristic of injured biliary canaliculi and of epithelium of the intestine where the remaining stubby microvilli often fuse together[38] (Fig. 1.8). In the small intestine, microvilli disintegrate into tiny vesicles, often in such large numbers that they may be confused with virions.

Cilia Disappear

Cilia become deformed, shortened, and disappear. Microtubular components retract and disassociate from other filaments in the cilium. Affected cilia may break or may retract with loss of internal components to the cytoplasmic matrix of the underlying cytoplasm. **Deciliation**, a common response of the respiratory tract, is typical of viral diseases such as influenza and paramyxovirus infections. Degeneration of the basal body that anchors cilia in the cell body usually precedes loss of cilia. Loss of cilia may be part of an active controlled process in the injured cell (rather than direct destruction by toxins). Some protozoa and algae cast off flagellae and other microtubule-containing components in response to injury. **Flagellar excision** involves severing of microtubules at specific sites near transition zones, special regions between flagellar axoneme and basal body, and is initiated by an increase in calcium in the cell.

Damaged Cytoplasm Is Shed by Exocytosis

Cytoplasmic Shedding

Cytoplasmic shedding is the exocytotic release of cellular material into the environment, either into interstitium or into lumens and ducts. Shed structures include:

- **Exosomes** (originating in microvesicles)
- **Membrane fragments**
- **Cytoplasmic blebs**

Cytosplasmic shedding is particularly active in surfaces bearing microvilli; for example, circulating leukocytes release procoagulant materials into the circulation by blebbing and cytoplasmic shedding. Cytoplasmic shedding is markedly increased in acute cellular injury, but also operates in normal cells in which it acts as a mechanism for release of small

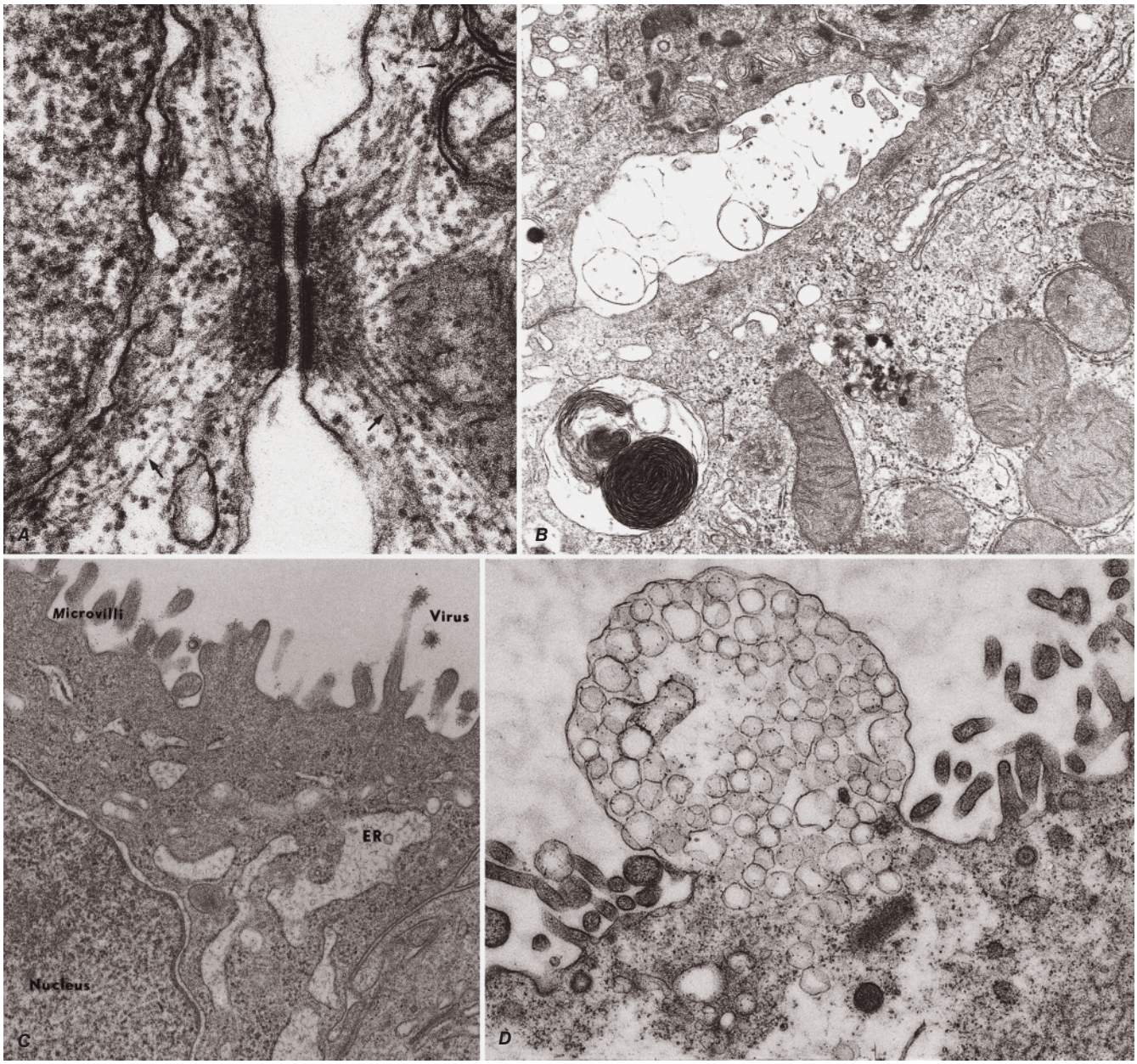

Fig. 1.8 Cell Surface pathology. A. Intercellular edema, persistence of desmosome: epidermis, fish. Intercellular filaments (*arrow*) radiate into the cytoplasm from submembrane plates of the desmosome. **B. Intercellular space expansion**: biliary canaliculus, liver, rat. Note increased thickness of intercellular junctions and loss of microvilli. **C. Surface structure degradation**: acute cell swelling, tracheal epithelium, paramyxovirus infection. Cisternae of the endoplasmic reticulum (*ER*) and nuclear envelope are dilated. Cilia are absent, and the cortical cytoplasm is devoid of basal bodies and microfilaments. Surface microvilli are irregular. Virions at the cell surface are labeled with ferritin (*Virus*). **D. Surface blebbing**: blebs, or bullae, on the surface of a pulmonary type I cell, lung, measles pneumonitis, monkey.

packets of damaged cellular components (see the section in chapter 5 on exocytosis).

Microvesicular Exocytosis

Small membrane fragments released as tiny **microvesicles** typically remain attached to the cell surface. In the kidney, microvesicles containing procoagulant material bleb from glomerular epithelial cells. Microvesicles are released into the lumen of the nephron and appear in urine;[39] an increase in microvesicles in urine is an index of renal injury.[40] Microvesicular shedding increases rapidly in injured renal tubules. Electron micrographs of renal tubular epithelium of rats poisoned with gentamicin reveal **membrane fragments** and dense **myelin figures** at the lumenal surfaces; micrographs of centrifuged urine show the same structures.[41]