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The Pathways of Cell Death: Oncosis, Apoptosis, and Necrosis*

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ABSTRACT

The pathways and identification of cell injury and cell death are of key importance to the practice of diagnostic and research toxicologic pathology. Following a lethal injury, cellular reactions are initially reversible. Currently, we recognize two patterns, oncosis and apoptosis. Oncosis, derived from the Greek word "swelling," is the common pattern of change in infarcts and in zonal killing following chemical toxicity, e.g., centrilobular hepatic necrosis after CCl4 toxicity. In this common reaction, the earliest changes involve cytoplasmic blebbing, dilatation of the endoplasmic reticulum (ER), swelling of the cytosol, normal or condensed mitochondria, and chromatin clumping in the nucleus. In apoptosis, the early changes involve cell shrinkage, cytosolic shrinkage, more marked chromatin clumping, cytoplasmic blebbing, swollen ER on occasion, and mitochondria that are normal or condensed. Following cell death, both types undergo postmortem changes collectively termed "necrosis." In the case of oncosis, this typically involves broad zones of cells while, in the case of apoptosis, the cells and/or the fragments are often phagocytized prior to their death by adjacent macrophages or parenchymal cells. In either case, the changes converge to a pattern that involves mitochondrial swelling, mitochondrial flocculent densities and/or calcification, karyolysis, and disruption of plasmalemmal continuity. The biochemical mechanisms of cell death are currently under intense study, particularly concerning the genes involved in the process. Pro-death genes include p53, the ced-3/ICE proteases, and the Bax family. Anti-death genes include ced-9/Bcl-2 and the adenovirus protein E1B. It is clear that ion deregulation, particularly that of $[Ca^{2+}]_i$ plays an important role in cell death following either apoptosis or oncosis. Genetic evidence strongly indicates that activation of proteases is an important step, possibly very near to the point where cell death occurs.

Keywords. Cell injury; intracellular calcium; ion regulation; volume regulation; gene expression; proteases

INTRODUCTION

The purpose of this paper is to analyze and interpret the cellular reactions to lethal injury, including those changes that occur prior to cell death and those that occur following cell death. Detection and accurate identification of such changes is important to toxicologic pathology because they may represent the earliest indicators of important toxic reactions to a variety of injuries including drugs, anti-microbiologic agents, toxic chemicals, and infectious agents. Definition of the criteria and the nomenclature of these processes is extremely important for purposes of data comparison and for experiments designed to elucidate the mechanisms of injury.

RESPONSES TO CELLULAR INJURY

Events Following Cell Injury: Terminology and History

The terminology in this field has had an ancient and colorful history and evolution in medicine. The past 10 yr has seen remarkable progress and, understandably, also some confusion in the literature concerning the terminology of the processes of cell injury and cell death (1,9,14,15,31).

Following injury, cells undergo a series of responses that collectively form what we recognize as a disease process. Many injuries to cells are sublethal and result in altered or new steady states in which the cells are able to survive in some altered or new steady state (26,29). Examples include vacuolization from dilatation of lysosomes and triglyceride accumulation. On the other hand, certain injuries, e.g., complete ischemia, are often lethal to cells and following a period of reversible reactions, the cell dies and then undergoes a series of degradative reactions that ultimately restore it to equilibrium with the environment. We can thus see that following a lethal injury there are a series of 3 phases: (1) changes that we term "prelethal," which are often reversible; (2) the "point-of-no-return" or cell death; and (3) postmortem autolytic and degradative changes. These postmortem cellular changes are termed "necrosis," which is an ancient word used to describe the changes that cells and tissues undergo following their death within a living organism. Not all types of death are followed by necrosis, e.g., tissues fixed in formaldehyde are the classic example of cells that are killed very rapidly but which subsequently do not undergo the degradative changes of necrosis.

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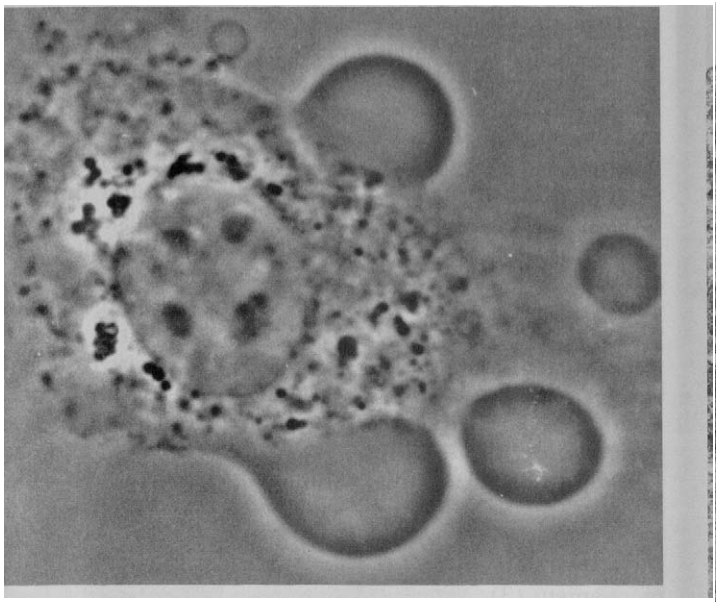

FIG. 1.—Phase micrograph of a JB6-clone 41 cell (promotable mouse keratinocyte cell line) following treatment with 10^{-4} M sodium lauryl sulfate for 45 min. Note the many blebs, marked vacuolization, and nuclear chromatin clumping all of which are reversible changes. ×1,700. [Reprinted with permission from Jain et al (12).]

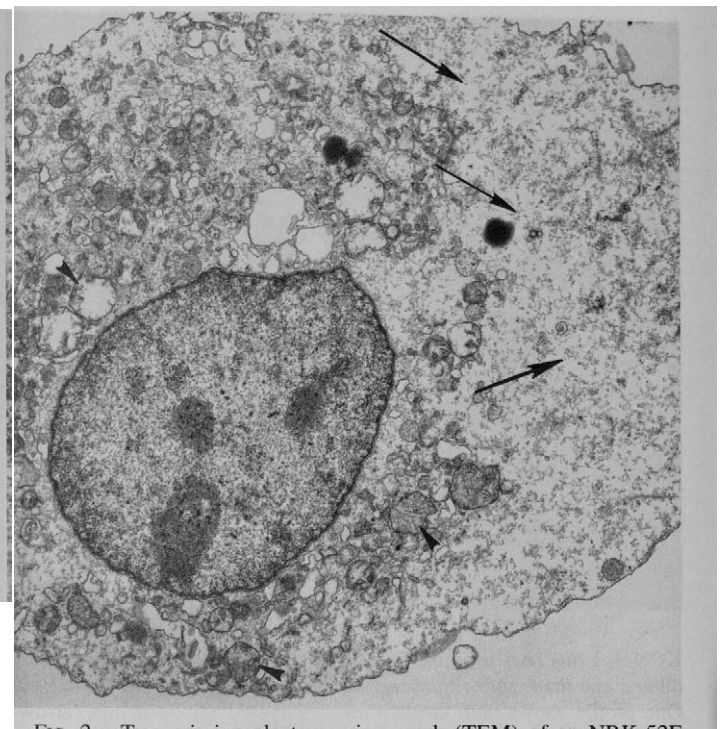

FIG. 2.—Transmission electron micrograph (TEM) of an NRK-52E cell (normal rat kidney cell line) treated with 1 mM KCN + 1 mM IAA (iodoacetic acid) for 3 hr, 45 min. Marked cytoplasmic swelling (arrows) can be seen in a large bleb region in which organelles and free ribosomes are absent. However, organelles are present in the nuclear region of the cell where most mitochondria are swollen (arrowheads). Cytoskeletal elements are not identifiable. ×8,000.

Clearly then, death and necrosis must be separated. At the same time, it is evident that it is illogical to speak of death by necrosis when in fact there are at least 2 different forms of necrosis occurring following death.

The terminology for the stages of cell reaction to injury prior to cell death has also undergone an interesting evolution over time. It turns out that there are 2 major categories consisting of the prelethal reaction which have come to be known as "apoptosis" and "oncosis." These are distinguished mainly on the basis of cell volume and nuclear morphology.

In apoptosis, the cells shrink, show multiple cytoplasmic protrusions or blebs, marked nuclear chromatin condensation, and ultimately fragmentation. Before this term was introduced by Kerr et al (13), many terms such as karyorrhexis, nuclear fragmentation, and individual cell necrosis were used to describe the phenomenon. On the other hand, another common prelethal type of reaction involves swelling of the cells prior to death. This had many terms over the years including vacuolization and cloudy swelling. Recently, Majno and Joris (15) proposed that we return to the term "oncosis," the term originally used in 1910 by von Recklinghausen (20). This, therefore, gives terms for both types of prelethal injury.

Another issue is the term "programmed cell death." During embryologic development in multicellular organisms, many cells die during the remodeling that occurs during the development of a variety of organs. Investigations of the genes involved in such cell death have revealed the existence of both pro- and anti-cell death genes. The mechanism of function of these genes is the subject of many current studies (4,7,10,11,23,30,32,33). However, at the same time, what is often overlooked is that every cell is totally programmed for death if deprived of a source of ATP or if the plasma membrane is violated.

The Concept of Programmed vs Accidental Cell Death

Recently, the concept of "accidental" vs "programmed" cell death has appeared often in the literature and, also, in our thinking. As far as we can determine, the concept of programmed cell death was originally derived from studies of developmental biology in which the death of cells in a particular area must die on schedule for embryologic development and organ differentiation to occur, such as normal phenotypes. Because this death in embryos is often (but by no means always) the apoptotic pathway, apoptosis has been erroneously equated with programmed cell death. It is very important to realize that every cell is "programmed" to die following an appropriate stimulus. The pattern, apoptosis or oncosis, clearly depends on both the cell type and the injury.

ONCOSIS

Morphology

In oncosis, the early changes include marked alterations in cell shape and volume, frequently occurring within seconds to minutes following application of the injury. In monolayer cultures, such cells form cytoplasmic blebs (19); chromatin clumping occurs later, followed by cells pulling apart, rounding up, and often detaching from the substrate (Fig. 1). *In vivo,* the cells often exhibit blebs,

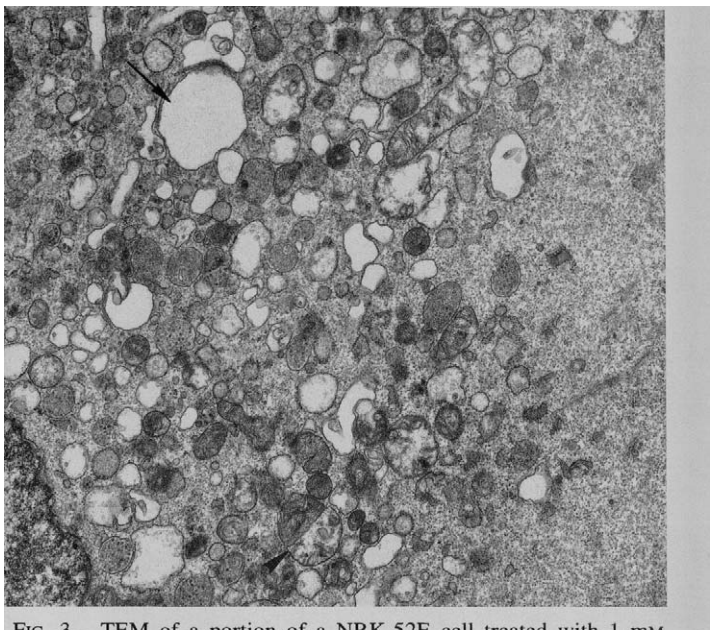

FIG. 3.—TEM of a portion of a NRK-52E cell treated with 1 mM KCN + 1 mM IAA for 3 hr, 45 min. The rough ER (arrow) is markedly dilated and many mitochondria are swollen while others are condensed. One profile shows both swollen and condensed compartments (arrowhead). On the right side of the micrograph, note the zone of swollen cytoplasm with free ribosomes and sparse organelles. At the top of the micrograph, the cell surface shows an irregular contour. ×16,000.

along the luminal borders and the vascular space, which later detach and, in the case of the kidney, form casts in the lumens of the nephrons. By electron microscopy, such cells show swelling and clearing of the cytosol, dilatation of the ER and Golgi, mitochondrial condensation followed by swelling, nuclear chromatin clumping, and multiple cytoplasmic blebs that typically are organelle-free (Figs. 2 and 3). During this phase, the elements of the cytoskeleton, including actin and tubulin, are markedly altered (8,18). When viewed with the naked eye, tissues undergoing this change appear opaque and cloudy, probably related to early and reversible denaturation of proteins. This is perhaps responsible for the use of earlier terms, such as "cloudy swelling."

Setting/Etiology

In vivo, oncosis typically affects broad areas or zones of cells, e.g., the early changes of cells following total ischemia or the cells in a particular region of the liver or kidney tubule following chemical toxins, such as CCl4, or HgCl2, respectively. When broad zones of cells are involved and following death of the cells, a pronounced inflammatory reaction typically occurs at the periphery of the zone. Although oncosis is not typical of programmed cell death in development, it has been described in some situations.

APOPTOSIS

Morphology

The earliest light microscopic changes include decreased cell volume, marked shape changes with multiple blebs and protrusions typically containing organelles, and marked nuclear chromatin clumping. By electron microscopy, the cytosol is dense, the ER may be dilated, the mitochondria are condensed and there is marked clumping of nuclear chromatin (Fig. 4). The nuclear outline becomes quite irregular with multiple protrusions that in section often appear to have detached, though they are frequently connected to the main nucleoplasm. At this stage, DNA strand breaks are often occurring that can be seen either in electrophoresis or with the deoxyribonucleotidyl transferase (TDT)-mediated dUTP–digoxigenin nick-end labeling (TUNEL) assay, although the latter is not specific for apoptosis. It is clear that apart from cell shrinkage, the morphologic changes are similar to those seen in oncosis. The main difference is shrinkage, apparently due to loss of ions, probably mainly K^+ and Cl^-. The significance of this volume alteration to the other changes, e.g., DNA strand breaks, needs further investigation; however, some experiments indicate that the shrinkage may indeed represent a significant step in the mechanism (3).

Setting/Etiology

Apoptosis occurs in a variety of settings. It is widespread during development and it is often in that situation that it actually represents a prelethal phase of programmed reaction to injury on a schedule set by various hormonal, nutritional, and micro- and macroenvironmental factors. In adult disease, it is commonly seen in situations with marked atrophy and regression where, in fact, the earliest descriptions of the process were made, including atresia of ovarian follicles, atrophy of the prostate following castration, and involution of the mammary epithelium following pregnancy.

Apoptosis also occurs following a variety of chemical and microbiologic injuries in many different organ systems (6,12,16,21,22,32,33). It is often difficult to estimate the extent, because of the rapidity with which the apoptotic cells and fragments are taken up by adjacent parenchymal and/or mesenchymal phagocytes. Although the inflammatory response is uncommon, presumably because the fragments are removed prior to death (24), Majno and Joris (15) have pointed out that there are some exceptions.

NECROSIS

Morphology

In the phase of necrosis, the changes are similar after either apoptosis or oncosis, though in some cases it appears that the cells showing apoptotic necrosis still remain somewhat shrunken or more dense, especially *in vivo.* As mentioned above, apoptotic necrosis typically occurs in apoptotic fragments that undergo this change within the phagolysosomal system of adjacent phagocytizing cells. *In vitro,* cells undergoing apoptotic necrosis (Fig. 4B) often show marked swelling and may be virtually indistinguishable from those showing oncotic necrosis. It is important to realize that either oncotic or apoptotic necrosis shows positive results with the TUNEL assay.

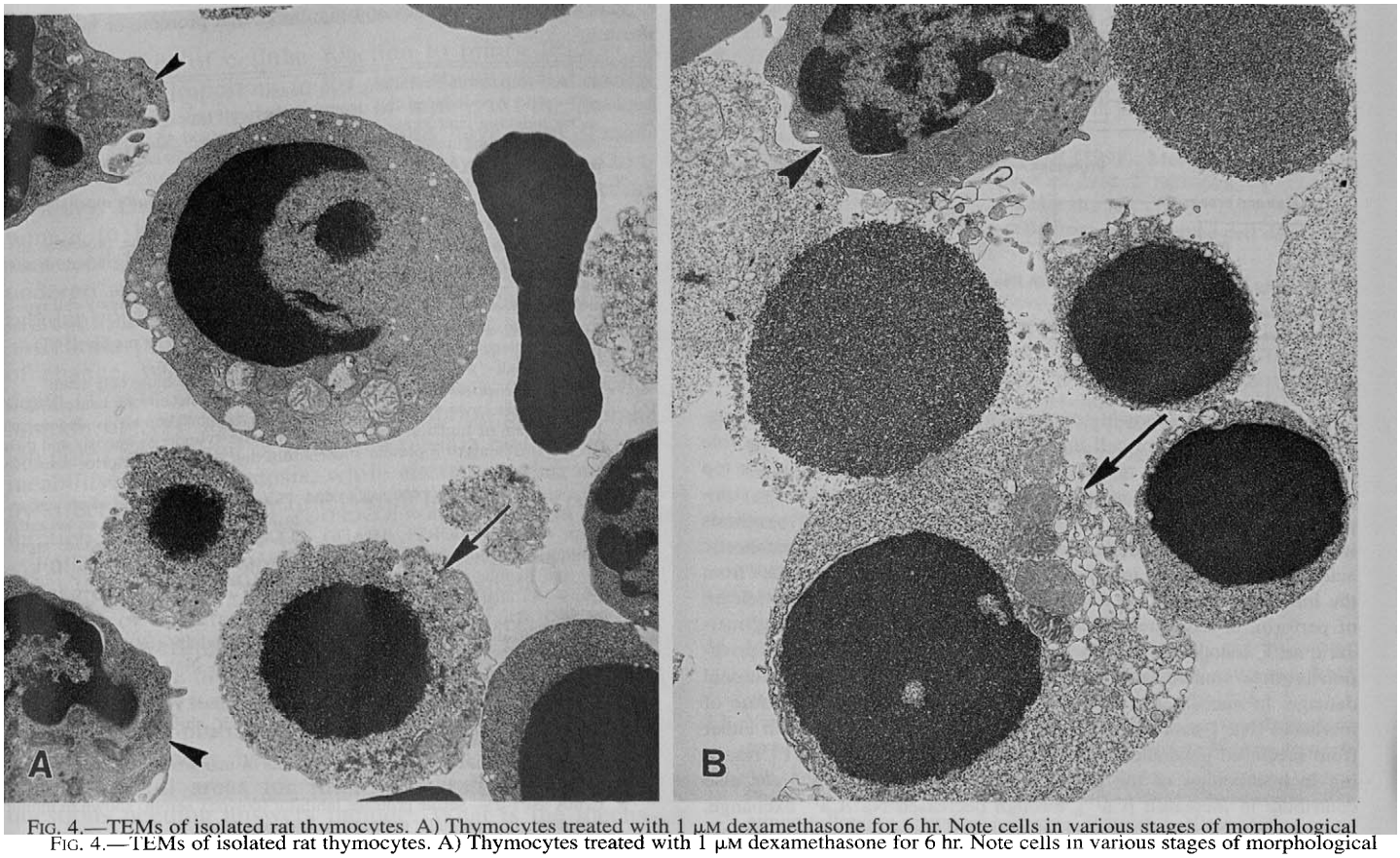

FIG. 4.—TEMs of isolated rat thymocytes. A) Thymocytes treated with 1 μM dexamethasone for 6 hr. Note cells in various stages of morphological changes. The cell in the center of the micrograph illustrates typical apoptotic ultrastructural changes, namely a nucleus that contains more than half condensed chromatin, cytoplasmic shrinkage, and relatively well-preserved organelle structure but with somewhat swollen mitochondria. Also illustrated is an apoptotic necrotic cell (arrow) and portions of cells that are near-normal (arrowhead). ×10,000. B) Thymocytes treated with 400 mM ionomycin for 6 hr showing a cell in the lower portion of the micrograph undergoing typical necrosis or cell death, most probably apoptotic necrosis (arrow). The 2 cells on the middle right portion of the micrograph show nuclei containing almost entirely condensed chromatin, while the cell in the upper left portion (arrowhead) is near-normal. ×10,000.

In both cases, it is our opinion that the most reliable signs of irreversible change are flocculent densities in the mitochondrial matrix and interruptions in plasmalemmal continuity, together with the formation of myelin figures. Meanwhile, the nucleus begins to show signs of degradation of the previously clumped chromatin, ultimately leading to karyolysis.

MECHANISMS/HYPOTHESIS

Understanding of the mechanisms of cell death is rapidly increasing. The goal of this research over the years has been to determine the sequence of biochemical events that leads to cell death following a variety of model injuries with the overall aim of determining the least number of pathways or possibly a final common pathway. This has been approached using a combination of morphologic, biochemical, physiological, and molecular biological methods.

ATP

A decrease in $[ATP]_i$ occurs very rapidly after many lethal injuries (2). This seems to be most important in situations that lead to oncosis as compared to apoptosis. In most mammalian cells, interference with ATP synthesis rapidly leads to de-energization of the Na^+,K^+-ATPase at the plasmalemma followed by an increase in $[Na^+]_i$ and $[Cl^-]_i$ accompanied by water influx and cellular swelling. As mentioned below, this is typically accompanied by a rapid increase in $[Ca^{2+}]_i$. This loss of volume control appears to be responsible for the marked swelling of the cell that typifies oncosis. In apoptosis, it may be that ATP levels are maintained longer during the reversible phase, precluding the inhibition of the Na^+ pump and cellular swelling. The shrinkage seen in apoptosis may play a role in the pathogenesis and may relate to the loss of K^+, accompanied by Cl^-, and stimulated by the increase in $[Ca^{2+}]_i$ and of Ca^+-activated K^+ channels.

Ion and Cell Volume Regulation

As mentioned above, oncosis is characterized by loss of volume control with massive swelling of most intracellular compartments, often based on ATP deficiency but also related to direct damage to the plasma membrane. In apoptosis, on the other hand, there is cellular shrinkage as one of the first morphologic effects. This implies loss of ions, presumably K^+ and Cl^-, as these predominate. As $[Ca^{2+}]_i$ is increasing during this period, we suggest that this K^+ loss is the direct result of an increase in

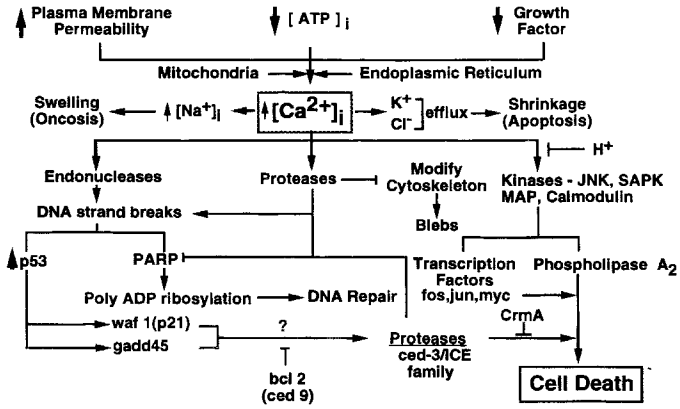

FIG. 5.—Diagram showing our current working hypothesis of the major events leading from cell injury to cell death, focusing on the role of $[Ca^{2+}]_i$. Some of the major categories of injury are shown at the top of the figure. Decreased and absent $[ATP]_i$ commonly results from complete ischemia, anoxia, or treatment with inhibitors of ATP synthesis including potassium cyanide, carbon monoxide, fluoride, and iodoacetic acid. Increased plasma membrane permeability commonly results from the immune response, e.g., activation of complement or T-cell release of perforin, or from treatment with a variety of toxins including mercuric salts, ionophores, or mechanical damage. Growth factor deprivation is often studied *in vitro* though, *in vivo,* it occurs with neuronal damage. In oncosis, the early events involve cell swelling because of increased $[Na^+]_i$ accompanied by Cl^- and water. This can result either from increased plasmalemmal permeability or decreased $[ATP]_i$ resulting in inactivation of the Na^+,K^+-ATPase. Increased $[Na^+]_i$ can also contribute to increased $[Ca^{2+}]_i$ through decreased Na^+/Ca^{2+} exchange. In apoptosis, the initial events include cellular shrinkage implying loss of ions, in particular K^+ and Cl^-. The mechanism of the shrinkage is not presently known although increased $[Ca^{2+}]_i$ might contribute through activation of K^+ channels. The initial increase of $[Ca^{2+}]_i$ can result from influx from the extracellular space or from redistribution from intracellular stores. Following the increase, several principal pathways seem to lead to reversible and irreversible changes. Also illustrated in the diagram are the events that follow Ca^{2+}-mediated activation of protein kinases, endonucleases, proteases, and phospholipases as discussed in the text. Our current hypothesis of altered gene expression related to cell death is shown near the bottom of the diagram. Abbreviations: ICE = interleukin-1β converting enzyme; JNK = jun N-terminal kinase; MAP = mitogen-activated protein; PARP = polyadenylate ribose polymerase [modified from Trump and Berezesky (28)].

$[Ca^{2+}]_i$ and stimulation of Ca^{2+}-activated K^+ channels. It may be that the shrinkage itself has some role in the pathogenesis of the changes.

Calcium

As indicated in the diagram depicting our current working hypothesis (Fig. 5), we, as well as other investigators, hypothesize that deregulation of $[Ca^{2+}]_i$ plays a pivotal role in both oncosis and apoptosis (5,21,22,25,27,28,32,33). Increased $[Ca^{2+}]_i$ stimulates a variety of signals and events that may involve virtually all aspects of cell behavior. One unsolved problem is why, under some conditions in some cells, increased $[Ca^{2+}]_i$ results in oncosis while, in other cells, it results in apoptosis.

Increasingly, the role of modulation of $[Ca^{2+}]_i$ in gene transcription and cytoskeletal function is also being elucidated.

TABLE I.—Selected genes and regulators that promote or inhibit cell death.[a]

A. Genes and Regulators Promoting Cell Death
1. p53—wild type induces cell death in many cell types; acts as transcription factor inducing later genes including waf/p21; gadd 45.
2. ced-3—gene originally described in the nematode *Caenorhabditis elegans*; encodes protease homologous to mammalian ICE.
3. ced-4—*C. elegans* gene; Ca^{2+}-binding protein whose precise mechanism of action is unknown.
4. ICE (interleukin-1β converting enzyme) family—cysteine proteases that can be classified into 3 groups: ced-3 subfamily; ICE subfamily; and NEDD-2 subfamily).
5. Fas/APO-1—a plasma membrane death receptor present in many cell types, which activates a killing pathway after binding to Fas ligand; related to the tumor necrosis factor family of receptors. Activation is mediated by signalling molecules such as FADD and TRADD.
6. Bax and Bak—members of the Bcl-2 family that promote cell death.
7. Degenerins—proteins in *C. elegans* found in somatosensory neurons; 2 of the 12 genes, mec-4 and mec-10, encode proteins known as degenerins because gain of function mutations cause cells to undergo oncosis and then necrosis. Belong to a protein superfamily that includes amiloride-sensitive Na^+ channels.
8. Reaper—*Drosophila* gene associated with apoptosis, which, when activated, leads to activation of ICE/CED-3 proteases; may represent an ancestral form of FADD/MORT1, TRADD, and RIP, which are molecules representing death domains that ultimately activate cell death.

B. Genes and Regulators Protective Against Cell Death
1. ced-9—*C. elegans* gene homologous to Bcl-2, which protects against cell death.
2. Bcl-2 family—includes Bcl-2 and Bcl-x_L which inhibit cell death and others such as Bax and Bak that promote cell death. May have domains that resemble membrane porins or diphtheria toxin.
3. Adenovirus protein E1B-19 kDa and Epstein Barr virus protein BHFRF-1—viral proteins that are counterparts of Bcl-2; inhibit protease activation and apoptosis induced by E1A.
4. p35—a baculovirus protein that functions in mammals, insects, and nematodes.
5. CrmA (SP1-2)—Serine protease inhibitor (cowpox serpin) shown to inhibit cell death in mammals, nematodes, and insects.
6. SPI-1—poxvirus serpin that inhibits poxvirus-induced apoptosis.
7. IAP (inhibitors of apoptosis) family—cell death suppressors first identified in viruses; baculovirus, *Drosophila,* and human homologs identified. The mammalian homologs, c-IAP-1 and c-IAP-2, are components of the tumor necrosis family receptor-2 (TNFR-2) complex that includes 2 associated factors TRAF-1 and TRAF-2.
8. NAIP—protein homologous to IAP identified in individuals with spinal muscular atrophy in which motor neuron apoptosis occurs in the absence of this inhibitor.
9. Dad1—death inhibitory protein—both mammlian and *C. elegans* homologs have been identified.

[a] Modified from Chinnaiyan and Dixit (4). For references, see those in Chinnaiyan and Dixit (4).

Genetic Regulation

In recent years, it has often become easier and more direct to identify the genes involved in a process than the precise biochemical mechanisms. This is eminently true for the changes that lead to cell death. A number of proteins have been found that can promote or protect against cell death [for review, see Chinnaiyan and Dixit (4)] (Table 1). The issue has evolved into attempts to discover the sequence of events and which ones are critical in a particular example. As knowledge of intracellular signalling pathways continues to develop, the pathways leading to cell death should be elucidated.

At the present time, it is not clear how a cascade of signalling events and proteins can lead to cell death, nor is it clear how some genes lead to apoptosis whereas others lead to oncosis. For example, in the nematode *Caenorhabditis elegans,* the genes ced-3 and ced-4 lead to apoptosis (34,35), whereas mutations in the degenerens gene lead to oncosis.

Discussion

The patterns of cellular reaction to injury leading to cell death are important in the understanding and recognition of toxic and other types of injury. At the present time, two types of prelethal reactions have been characterized: apoptosis and oncosis. Both may result from toxic injury. The factors that determine which process occurs remain to be identified. It does appear, however, that some cells such as lymphocytes, are particularly likely to undergo apoptosis while others, such as renal proximal tubular epithelium, seem especially prone to oncosis.

Cellular proteases seem to be involved in both types of change, particularly in the final stages prior to cell death (17). It appears that ATP deficiency, especially inhibition of ATP synthesis, and disruption of membrane ion pores or creation of increased plasma membrane permeability result in oncosis, while alterations that primarily affect DNA structure often result in apoptosis possibly through activation of p53.

Following cell death, apoptotic or oncotic cells undergo necrosis. At this point, the changes rapidly converge with loss of volume control in the case of apoptosis and continued swelling in the case of oncosis. In the case of apoptosis, these final stages usually occur within the phagolysosomal system of adjacent cells that have phagocytized the apoptotic cells and fragments.

The differences between oncosis and apoptosis are clearly fruitful areas for future research. For example, questions needing answers include: What is the mechanism by which p53 induces apoptosis and whether or not in some cases it could induce oncosis? What is the role of proteases and how does it differ in the 2 cases? What is the role of cellular shrinkage in initiating apoptosis? What is the final "execution step" and does it differ in the 2 cases? When these answers become known, the mechanisms and pathways of cell injury and cell death will then also become known, and an understanding of these events will lead us to better methods in diagnostic and research toxicologic pathology.

Acknowledgments

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ACVP UPCOMING MEETINGS

Annual Meetings

November 16–21, 1997	Hyatt/Doubletree, Albuquerque, NM
November 15–20, 1998	Adams Mark, St. Louis, MO
November 14–19, 1999*	Palmer House, Chicago, IL

Examinations
(always held in Ames, IA)

September 24–25, 1997
September 16–17, 1998
September 22–23, 1999
September 20–21, 2000
September 19–20, 2001
September 25–26, 2002

* 50th Anniversary of ACVP.