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Review

Apoptosis, Oncosis, and Necrosis

An Overview of Cell Death

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The historical development of the cell death concept is reviewed, with special attention to the origin of the terms necrosis, coagulation necrosis, autolysis, physiological cell death, programmed cell death, chromatolysis (the first name of apoptosis in 1914), karyorhexis, karyolysis, and cell suicide, of which there are three forms: by lysosomes, by free radicals, and by a genetic mechanism (apoptosis). Some of the typical features of apoptosis are discussed, such as budding (as opposed to blebbing and zeiosis) and the inflammatory response. For cell death not by apoptosis the most satisfactory term is accidental cell death. Necrosis is commonly used but it is not appropriate, because it does not indicate a form of cell death but refers to changes secondary to cell death by any mechanism, including apoptosis. Abundant data are available on one form of accidental cell death, namely ischemic cell death, which can be considered an entity of its own, caused by failure of the ionic pumps of the plasma membrane. Because ischemic cell death (in known models) is accompanied by swelling, the name oncosis is proposed for this condition. The term oncosis (derived from ónkos, meaning swelling) was proposed in 1910 by von Recklinghausen precisely to mean cell death with swelling. Oncosis leads to necrosis with karyolysis and stands in contrast to apoptosis, which leads to necrosis with karyorhexis and cell shrinkage. (Am J Pathol 1995, 146:3–15)

Knowledge in the field of cell death has greatly increased during the past 20 years or so. In the course of this rapid advance, new concepts, such as apoptosis, appeared on the scene, and ancient terms such as necrosis came to be used in a new context. Inevitably, some conceptual and semantic strains developed; a recent reviewer saw fit to conclude that "there is no field of basic cell biology and cell pathology that is more confusing and more unintelligible than is the area of *apoptosis versus necrosis*."[1] The purpose of this paper is to offer a critical and, we hope, constructive overview of the terms and concepts related to cell death.

It will be useful to begin by tracing the main steps that led us to where we now stand.

Development of the Cell Death Concept

The fact that cells can perish is discussed in Lecture XV of Virchow's Cellular Pathology among "passive processes and degenerations."[2] Understandably, no microscopic description of cell death is included, as histological stains were not used in 1858. Thus, topics related to cell death are treated in this lecture at a gross level, under names such as degeneration, softening, necrosis, and mortification, which was synonymous with gangrene. For our present purposes it is relevant to note that Virchow uses necrosis to mean an advanced stage of tissue breakdown, similar to what we would now call gangrene: "In necrosis we

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We dedicate this paper to the memory of Dr. Marcel Bessis, pioneer in the study of cell death, eminent clinician, and founder of the first Institute of Cellular Pathology, who passed away in Paris on March 28, 1994.

conceive the mortified [gangrenous] part to be preserved more or less in its external form" (p. 358). This had been the traditional usage of the term necrosis, not only in the textbook of Virchow's teacher Rokitansky[3] but also, as *nécrōsis*, in ancient Greek texts at least since Galen.[4]

Virchow's Lecture XV also creates a special category of regressive processes under the name **necrobiosis,** a term he borrowed from a contemporary author, K. H. Schultz. Included among the necrobiotic processes, for example, were the "softenings." Necrobiosis is thus defined, perhaps, to fit the model of brain softening: "The part vanishes, so that we can no longer perceive it in its previous form. We have no necrosed fragment at the end of the process." A footnote extends the definition (emphasis original): "Necrobiosis is *death* brought on by (altered) *life* - a spontaneous wearing out of living parts - the destruction and annihilation consequent upon life - natural as opposed to violent death (mortification)." The use of necrobiosis throughout Virchow's book and later indicates that this term was sometimes meant to imply also "slow death" or "death of tissues within the living body." Needless to say, necrobiosis was a vague and ambiguous term. After a long career it is finally disappearing. Another term from Virchow's Lecture XV that needs rethinking is **degeneration.** Virchow's "fatty degeneration" still clings on[5]; it should be banished, because cells can die but certainly cannot degenerate into something else. In our opinion, there are few defensible uses of this term in general pathology; we can think of two: for the breakdown of axons, as in Wallerian degeneration, and for the breakdown of cartilage matrix in degenerative osteoarthritis.

The next step, around 1877, was the identification of **coagulation necrosis** by Carl Weigert and Julius Cohnheim. Today this term evokes a rather obvious and noncontroversial lesion, mainly the white infarct, but this was not true in the beginning, when it applied to a condition that in our view has virtually nothing to do with coagulation necrosis.

When Carl Weigert (1845–1904) was training under Virchow, diphtheria was a common cause of death, as the vaccine was not available before 1880. Diphtheria causes necrosis of the tracheobronchial epithelium, which becomes impregnated with fibrin and leukocytes and tends to slough off as a leathery, whitish pseudomembrane (*diphthéra* is Greek for tanned hide). Weigert, who was especially interested in fibrin (witness the fibrin stain of his name), became impressed by this combination of epithelial necrosis and fibrin.[6–9] He held the current view that dead leukocytes cause fibrinogen to coagulate and proposed that the same mechanism that produced the diphtheritic pseudomembrane was at work also in producing what we now call white infarcts. He even referred to the typical wedge-shaped white infarcts as fibrin wedges (*Fibrinkeile*).[9] Julius Cohnheim, another disciple of Virchow, accepted this view and introduced, in the 1877 edition of his textbook, the term coagulation necrosis.[10] Soon the fibrin component was found to be unrelated to the coagulation of the tissue[11]; today Weigert's concept survives only in what we call fibrinous necrosis. However, an important byproduct of Weigert's studies was the observation that necrotic cells lose their nuclei.[6]

The role of protein denaturation in the genesis of coagulation necrosis was vaguely hinted in Cohnheim's textbook[10]; it could not escape the pathologists' attention that necrotic tissue looked like "coagulated albumen."[12] Interestingly, the notion that protein denaturation may participate in cell death was first proposed in 1886 by a botanist, G. Berthold.[13,14] This is just one of the many contributions made by plant pathology to animal pathology, the latest one being the tetrazolium method for detecting dead tissue on gross specimens, originally used by botanists for identifying nonviable seeds (reviewed in reference 15).

Weigert's notion of coagulation necrosis had the virtue of triggering, after 1880, many experimental studies on cell death,[16] produced by ligating the renal artery, by maintaining tissue fragments septically or aseptically *in vitro*,[11] or by introducing them into the peritoneal cavity of experimental animals.[17] Much of the present terminology of cell death, besides coagulation necrosis, stems from that era. **Autolysis** was proposed in 1900, although the concept was known earlier.[13,16,18] **Pyknosis** was in use around 1890.[19] **Karyolysis** and **karyorhexis** were proposed in 1879[20] by Edwin Klebs of Klebsiella fame (he spelled his Karyorhexis right, but the erroneous Karyorrhexis appeared in the title of an 80-page paper in 1890[19] and has lasted ever since). Chromatin margination (Randstellung) was described in 1890.[17] Some terms born in those days changed meaning, such as chromatolysis (1885),[21] and others disappeared, such as plasmarhexis,[20], chromatopyknosis, and deconstitution.[22]

Spontaneous cell death as a physiological event was discussed almost as soon as stains became available. It was born with a bang in 1885 in a paper[21] by the same Walther Flemming who created the terms chromatin and mitosis.[23] Flemming studied ovarian follicles in mammals and noticed that the epithelial lining of regressing follicles was littered with cells the nuclei of which were breaking up (Figure 1).[21] His careful *camera lucida* drawings illustrate the half-

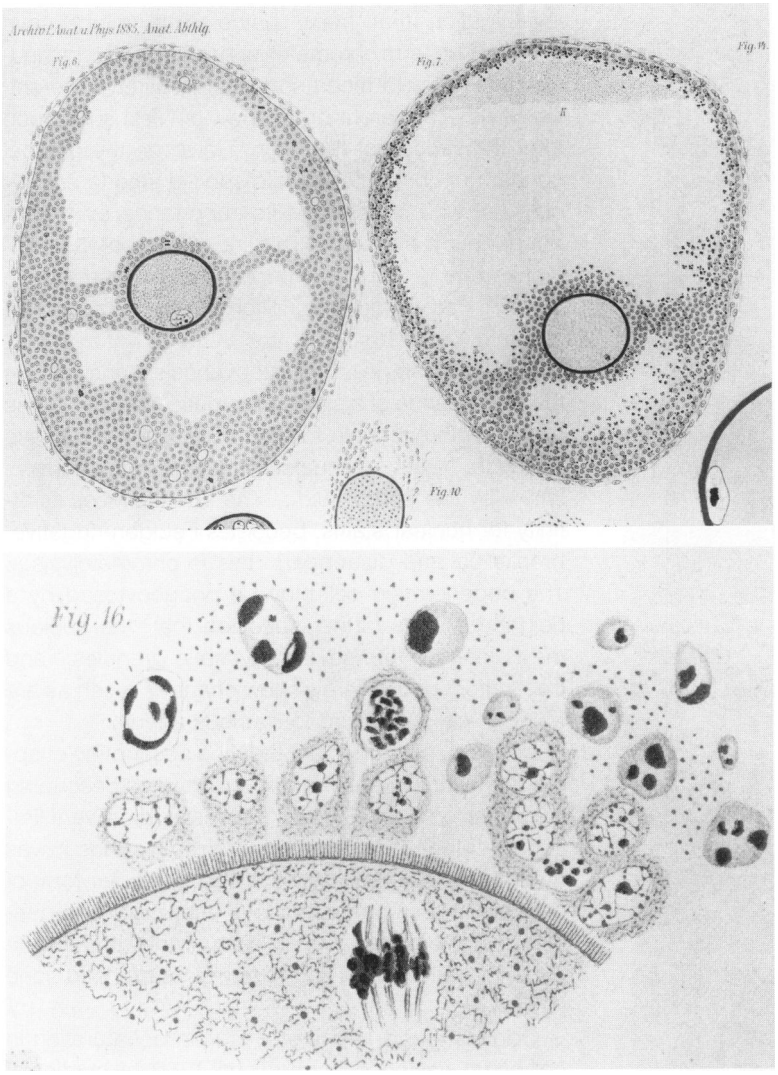

Figure 1. *Apoptosis as observed in 1885 by Flemming,[21] who called it chromatolysis.* **Top left:** *Normal rabbit ovarian follicle near maturity, 1 mm in diameter. Numerous epithelial mitoses.* **Top right:** *Early stage of involution in a nearby follicle. Many epithelial cells are in various stages of death by chromatolysis; some are shed into the lumen.* **Bottom:** *detail of the same involuting follicle. Most epithelial cells in contact with the ovum are normal (one is in mitosis); those farther removed are undergoing chromatolysis. Note the half-moons of chromatin typical of apoptosis. (Osmium fixation; safranin and gentian violet staining. Camera lucida drawings.)*

moons of pyknotic chromatin typical of apoptosis (Figure 1) as well as apoptotic bodies loose in the cavity of the follicle. Flemming gave a name to the process, **chromatolysis,** referring to the fact that the broken up nucleus ultimately disappears. A few months later the same observations were published by a German medical student, Franz Nissen,[24] who observed chromatolysis also in lactating mammary glands (Figure 2).

Chromatolysis and nuclear pathology became a fashionable topic[25,19]; beautiful examples of what we would label apoptosis were seen in breast cancers by Ströbe,[26] and by 1914 enough data were available for a German anatomist, Ludwig Gräper, to publish a paper entitled (in translation) "A new point of view regarding the elimination of cells."[27] Gräper's premise is that some mechanism must exist to counterbalance mitosis, especially in epithelia, and concludes that Flemming's chromatolysis is the answer: "Chromatolysis must exist in all organs in which cells must be eliminated" (p. 377). The debris, he writes, are taken up by neighboring epithelial cells, but sometimes they are so abundant that they are eliminated into an organ's lumen, as is the case for "uterine milk" (both features are typical of what we now call apoptosis). Gräper also experiments on the yolk sac, which, he argues, must shrink progressively: its cells do not shrink, so the sac as a whole could only become smaller by one of two mechanisms: 1) by developing folds or 2) by eliminating cells, which Gräper recognizes as the right answer (Figure 3). Gräper concludes that the "physiologische Zellelimination" occurs by chromatolysis during the shrinkage of organs, as well as normally in certain glands. In essence, "a sister cell [*Schwesterzelle*] engulfs a neighboring cell that breaks down" (p. 391). An interesting side issue comes up as Gräper points out that the persistent descriptions of so-called amitosis (nuclear division

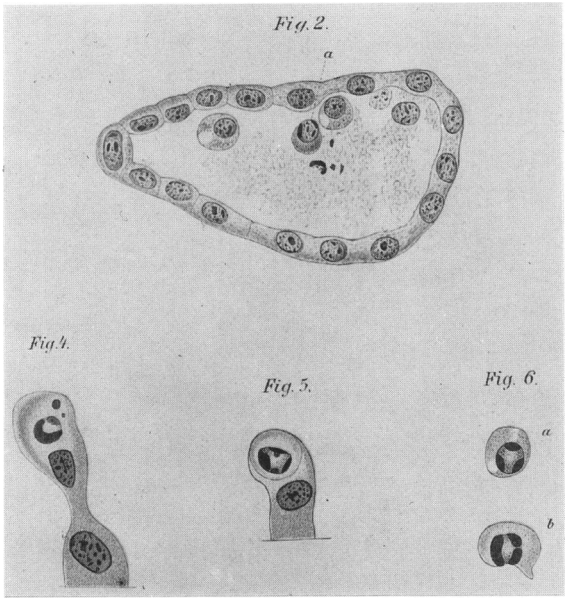

Figure 2. *Apoptosis as seen in 1886 by a German medical student, Franz Nissen, in the lactating mammary gland.[24] Nissen became aware of Flemming's study,[21] after having completed his own, and concluded that the name chromatolysis was very suitable also to his own findings.*

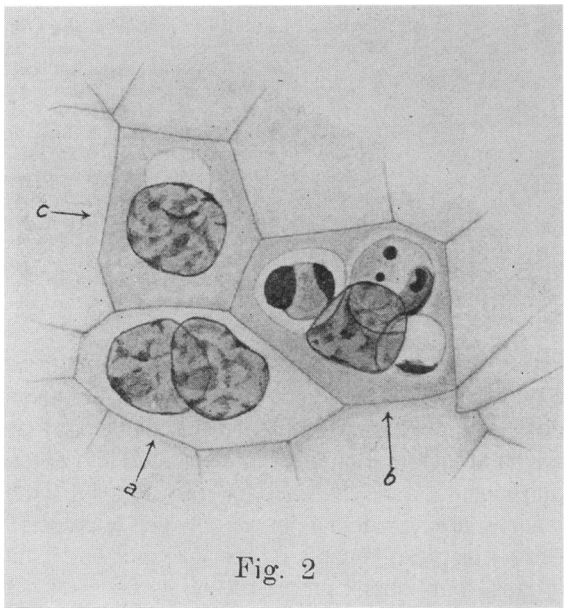

Figure 3. *Apoptosis illustrated in 1914 by L. Gräper as chromatolysis.[27] Wall of the yolk sac in the course of involution, in a 20-cm embryo of* Acanthias, *a creature that we have been unable to identify. An epithelial cell has taken up the fragmented nucleus of a neighboring cell that died during the involution process.*

without mitosis) were due to the erroneous interpretation of cells that had taken up nuclear material from a nearby cell that died by chromatolysis.

This milestone paper made no significant impact. Perhaps it was overlooked because it appeared at the outset of World War I in a German journal on cellular investigation that many pathologists might have missed. The term chromatolysis was adopted by neuropathologists to mean something entirely different, namely the apparent breakdown of Nissl substance after transection of the axon. However, the original concept of chromatolysis did survive among embryologists, who understood its importance as a morphogenetic mechanism. This line of studies was summarized in a masterly paper by Glücksmann in 1950.[28] Here is his description of physiological cell death in the embryo.

"The initial stage, chromatopycnosis, consists ... in the appearance of a single chromatic mass sitting as a cap on the vacuole formed by the non-chromatic material.... Both the nucleus and the cytoplasm .... shrink by the loss of fluid.... The granule loses its affinity for nuclear stains, becomes Feulgen-negative, breaks up and disappears: this is chromatolysis.... The degenerating cell may be phagocytosed by a neighbour." It is further specified that "the nucleus may break up into several pycnotic granules," and that "mitochondria rarely show changes" such as are found in cells exposed to injurious agents.

All this is, of course, another fine description of apoptosis. Glücksmann realized that he was describing a special form of cell death, but his studies were limited to cell death in embryonic tissues. Thus it was natural for him to assume that this particular form of cell death was characteristic of vertebrate ontogenesis and therefore different from, rather than applicable to, cell death in adult tissues. Gräper had gone farther.

Oddly enough, the role of protein denaturation in cell death was not confirmed until 1960, by studies of optical density, light diffraction, and autofluorescence of dying and dead tissues.[29] These studies were carried out by the old method of implanting fragments of rat liver into the peritoneum of other rats. The following appear to be the basic rules of ischemic cell death for the liver; variations should be expected for other cell types:

1) Cell death and necrosis are very different entities. Ischemic cell death, defined functionally by the point of no return, occurs long before necrosis and is not detectable histologically (for rat liver the point of no return is known to occur at approximately to 2 to 2.5 hours); indeed, it is useful to point out that the difference between cell death and necrosis, biologically a key issue, has been completely overlooked in recent literature.[2]

2) Ischemic liver cells swell for 6 to 7 hours, then lose water and shrink (Figure 4). Early in the swelling process they die. We can now add that the swelling process is understood as a result of ion pump failure

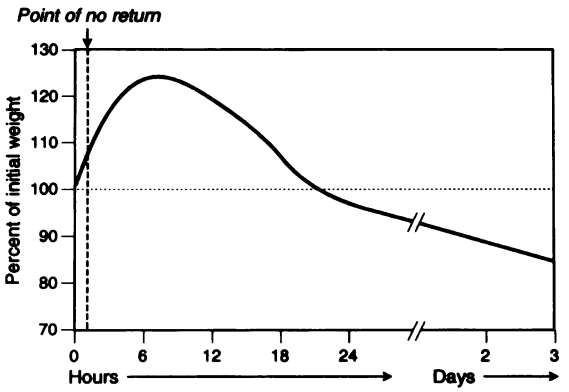

Figure 4. *Curve obtained by weighing fragments of rat liver after they had been left in the peritoneal cavity of other rats for 0 to 72 hours. It shows that ischemic liver cells swell before they die, continue to swell thereafter, then shrink. The approximate time of cell death is indicated as the point of no return. Data from Majno et al.*[29]

by lack of ATP and that the swelling is accompanied by intense blebbing (to be discussed further).

3) The dying and dead cell's proteins face two possible fates: hydrolysis and/or denaturation.

4) Protein denaturation begins while the cell is still alive, being detectable at 30 minutes. Today we can interpret this finding by assuming that the ubiquitin system, which targets denatured cells for hydrolysis by an energy-requiring system,[30,31] is overwhelmed and can no longer function for lack of ATP. The denaturation process is still going on after 12 days.

5) During ischemic cell death and the subsequent coagulation necrosis, large amounts of calcium are taken up by the affected cells.[32]

The concept of cell suicide surfaced for the first time after the lysosomes were discovered in the late 1950s. De Duve proposed that cells might be killed from within, by an explosion of their lysosomes acting as "suicide bags" (summarized in reference 15). The idea was soon discredited, but we now know that it is probably true in special circumstances, namely in the crystal diseases, in which leukocytes phagocytize crystals that break open the lysosomes (summarized in reference 15).

Free radical pathology appeared in the 1960s, and it led to the identification of another mechanism of cell suicide, especially in liver cells as a result of certain intoxications,[33] namely, the intracellular release of free radicals, which can damage cellular organelles (summarized in reference 15). This line of research helped lead to the proposal of a final common pathway for cell death from different causes, ie, a rise in intracellular calcium.[34,35]

Then came apoptosis, the third and perhaps the ultimate form of cell suicide, purposeful suicide, and one of the most exciting developments in modern biology. The critical experiment (published in 1971) was extraordinarily simple; Kerr[36] induced liver atrophy in the rat by tying off a large branch of the portal vein. He noticed a discrete drop-off of cells by a sequence of changes that he called at first shrinkage necrosis and a year later apoptosis.[37] The next critical step came independently from the study of irradiated lymphoid tissues. It was known from histological studies that the nuclei of irradiated lymphocytes break down.[38] In 1976[39] and 1981[40,41] three groups examined electrophoretically the chromatin of irradiated tissues and found that it broke down into fragments that produced a typical, ladder-like pattern, suggesting that the fragments were multiples of nucleosomes.[41] Then Wyllie et al[42] in 1984 linked the ladder pattern with the phenomenon of apoptosis and thereby added a specific biochemical marker to the distinctive morphological changes of apoptotic cells. This discovery led to an enormous increase in papers on apoptosis, with the latest developments in this field concerning the genes involved in cell suicide and the possibility of using apoptosis as an approach to tumor diagnosis and therapy.[43–47]

In retrospect, it is mind-boggling that earlier pathologists (ourselves included) paid so little attention to the mechanism of organ shrinkage during atrophy, even after it had been carefully described. Perhaps it appeared too simple and self-evident, or as dull as autolysis *in vivo*.[48] It is a fair guess that today the mechanism of atrophy would have a low priority in competing for grant support.

We will now examine more closely the two best known modalities of cell demise: cell death by suicide (apoptosis) and cell death by murder (accidental cell death).

Cell Death by Suicide: Apoptosis

As mentioned earlier, cells can commit suicide by at least three mechanisms, but apoptosis stands out as a form of intentional suicide based on a genetic mechanism. For our purposes it will suffice to list the key features of apoptosis, as many comprehensive reviews are available.[49–52]

1) Apoptosis is a form of cell death characterized by morphological as well as biochemical criteria and can be considered as a counterpart of mitosis, as Gräper had proposed.

2) Morphologically the cell shrinks and becomes denser, as implied in the original name shrinkage necrosis.[36] The chromatin becomes pyknotic and packed into smooth masses applied against the nuclear membrane (margination of chromatin; Figure

5), creating curved profiles that have inspired descriptive terms for over a century, such as half-moon-, horse-shoe-, sickle-,[19] lancet-, and ship-like (navicular[30]). The nucleus may also break up (karyorhexis), and the cell emits processes (the budding phenomenon) that often contain pyknotic nuclear fragments. These processes tend to break off and become apoptotic bodies, which may be phagocytized by macrophages or neighboring cells or remain free; however, the cell may also shrink into a dense, rounded mass, as a single apoptotic body.

3) There is little or no swelling of mitochondria or other organelles.

4) Biochemically, the DNA is broken down into segments that are multiples of approximately 185 bp, due to specific cleavage between nucleosomes.

5) The process is under genetic control[46,47] and can be initiated by an internal clock, or by extracellular agents such as hormones, cytokines, killer cells, and a variety of chemical, physical, and viral agents.

6) Apoptosis can run its course very fast, even in minutes (34 minutes from the onset of budding to complete breakup in the movie by Bessis to be discussed below). For this reason apoptosis is remarkably unobtrusive in tissue sections.[53] In routine sections the best cytological marker of apoptosis is karyorhexis, especially in an isolated cell. Fortunately, a recent technical advance makes the identification of apoptosis a matter of simple histochemistry, a method that takes advantage of the fact that the DNA breaking points (nicks) expose molecular endings that are chemically specific.[54]

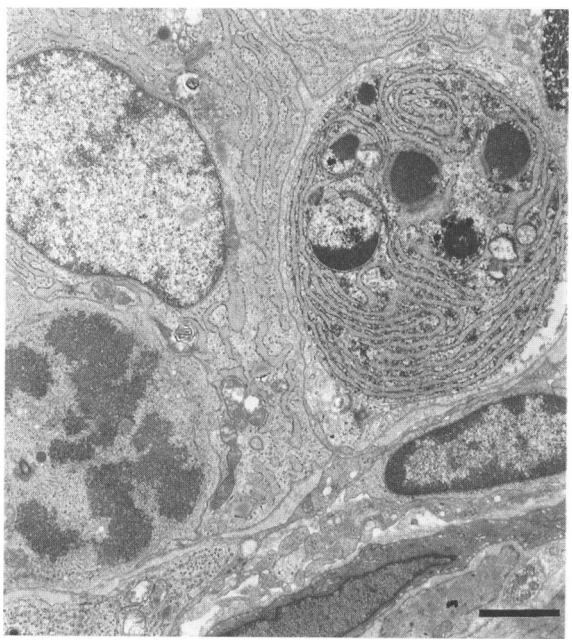

Figure 5. *The two main regulators of cell populations, mitosis* (bottom left) *and apoptosis* (top right). *In the apoptotic cell, the nucleus is fragmented* (*karyorhexis*) *and the chromatin is pyknotic. One of the nuclear fragments contains a characteristic half-moon of condensed chromatin.* (*Electron micrograph from a rat prostate 2 days after castration. Bar = 2 μ*).

7) The rapidly developing tale of apoptosis warns us that generalizations are dangerous because, first, cell suicide does not always take the form of apoptosis; second, cell murder by cytotoxic lymphocytes leads to apoptosis; third, there seem to be several varieties of apoptosis[55] and fourth, different cell types may follow different rules.[2]

The only flaw that we find in the name of apoptosis is that it includes both cell death (presumably represented by cell shrinkage and pyknosis) and necrosis (the secondary breakup into a cluster of apoptotic bodies). This has created some confusion: how can apoptosis be opposed to necrosis, as many authors do, if apoptosis produces classic images of necrosis? The remedy may be simple enough: the second phase of apoptosis should be called *apoptotic necrosis*, as opposed to ischemic, toxic, or massive necrosis (see below). Examples of apoptotic necrosis are the sunburn cells of the epidermis and the Councilman bodies of the liver.[53]

We might add that the Greek name apoptosis is most felicitous, suggesting as it does the discrete image of leaves dropping off here and there from a tree (*apó*, meaning from and *ptósis*, meaning a fall) as opposed to the massive cell death of an infarct. We will only note that the pronunciation *apo'tosis* (skipping the second *p*)[37] is not confirmed by the Greek scholars whom we have consulted. A recent letter to *Nature*[56] makes the same point: nobody says helico'ters.

The Shrinkage and Condensation of Apoptotic Cells

It is interesting to compare these two features of apoptosis with the shrinkage and condensation that occur after ischemic cell death as a result of protein denaturation[23] (Figure 4). To this day, the shrinkage and condensation of the apoptotic cell are not explained.[46] Could they be rooted in the same mechanisms that operate in ischemic cell death? The increased density, visible on light and electron micrographs, could reflect the accumulation of denatured proteins, perhaps by failure of the ubiquitin system. Protein denaturation has been linked to increased intracellular calcium,[34,35] and in some forms

of apoptosis calcium does increase.[46,56] Because denatured proteins are autofluorescent, their presence in apoptotic cells should be fairly easy to test, and we are presently attempting to do so.

The Budding Phenomenon

In their agony, cells dying by apoptosis emit a number of pseudopodia, a process that Kerr has aptly described as budding.[50] The emission of cellular processes, obvious on electron micrographs of apoptotic cells,[50] becomes dramatic when witnessed by time-lapse cinematography of isolated cells. We can say this because apoptosis was filmed accidentally in the 1950s, during a study of cell death,[58–60] well before apoptosis was recognized as a special entity. A French hematologist, the late Marcel Bessis, examined by time-lapse cinematography the modes of cell death of human leukocytes maintained between slide and coverslip (Figure 6). One of the sequences shows a leukocyte emitting pseudopodia (budding) and finally breaking up with almost explosive suddenness. Bessis described this cellular behavior as "cell death by fragmentation."[60] A study frame by frame even shows two half-moons of chromatin in an apoptotic body (Figure 6). The sequence was seen by Dr. Kerr who agreed that it appears to represent apoptosis (JFR Kerr, personal communication, 1994).

The pathogenesis of the budding phenomenon is not understood; perhaps it is related to the final breakup of the cell. It is milder in the stiff keratinocytes,[53] perhaps explaining why apoptosis of keratinocytes can produce the relatively large, rounded intraepithelial bodies called sunburn cells.[53] The buds may contain any type of organelles, including nuclear fragments, and do not swell; they should not be confused with blebs. Blebs are typical of ischemic cell death. They are blister-like, fluid-filled structures, typically devoid of organelles, that arise from the cell membrane and are apt to swell and burst, and some may pinch off and float away. Some blebs are reversible. The mechanism of blebbing appears to depend on a disconnection between the cell membrane and the underlying cytoskeleton (reviewed in reference 15).

Students of apoptosis sometimes refer to the dying cell as performing zeiosis.[52,53] The term zeiosis (from the Greek *zéiō*, meaning I boil) was created by Costero and Pomerat in 1951[61] to describe a bubbling process observed in living cultured fragments of nervous tissue. The bubbling occurred along dendrites and was not studied in detail, but it seems to have represented blebbing. To our knowledge, blebbing has never been seen in electron micrographs of apoptotic cells. Therefore, in the context of apoptosis, it is best to use the term budding instead of zeiosis.

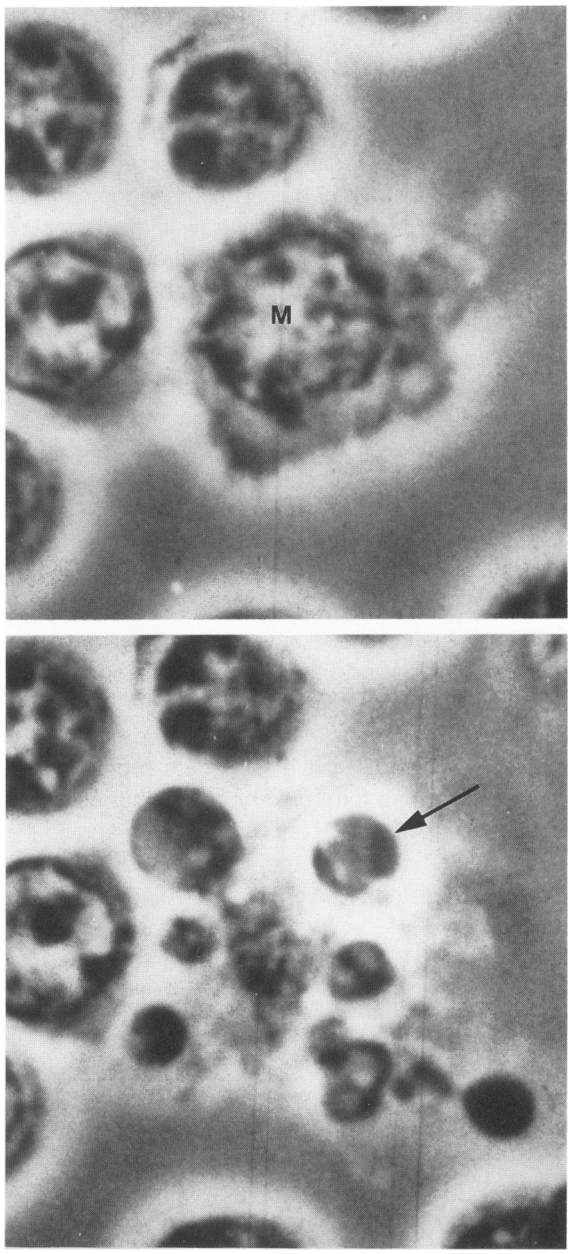

Figure 6. *The first known cinematographic recording of apoptosis. Two frames from a 1955 time-lapse movie by Marcel Bessis, showing a leukocyte* in vitro *said to be dying "by fragmentation."*[57–59] **Top:** *A leukocyte (probably a monocyte, M) just before its demise.* **Bottom:** *The same leukocyte 33 minutes later, after an episode of budding (not shown). It has suddenly broken up into apoptotic bodies, two of which contain clumps of dense chromatin (arrow), most likely the typical half-moons of apoptosis. (Reproduction authorized for Dr. M. Bessis by Dr. J. L. Binet.)*

Apoptosis and Karyorhexis

Karyorhexis, which used to be a descriptive term of little relevance, has gained new status since it turned out to be a feature of apoptosis. It is true that karyorhexis, when observed in isolated cells, suggests apoptosis. However, it is certainly not pathognomonic of apoptosis. Its originator, Klebs, saw it in a variety of dying (and infected) tissues.[20] Neurons can show karyorhexis as a result of ischemia[62] and hyperoxia.[63] Karyorhexis occurring in tumors might be taken as a manifestation of programmed cell death and, therefore, as a good prognostic sign, in opposition to the number of mitoses (and to the extent of massive necrosis).[64] However, the literature in this regard is somewhat confusing. Some authors interpret karyorhexis in malignant tumors in the same way as massive necrosis, that is, as a bad sign. Because many mitoses are also an indication of poor prognosis, karyorhexis and mitosis have been lumped together as a mitosis-karyorhexis index, used to mean "number of nuclei showing either mitosis or karyorhexis per high power field."[65] When this is done, frequent karyorhexis correlates with poor prognosis, at least for neuroblastoma. Is this a misunderstanding, or are we dealing with a form of karyorhexis that does not represent apoptosis? This puzzling issue should be easily settled by using the specific histochemical stain,[54] rather than karyorhexis, for diagnosing apoptosis.

Apoptosis and Inflammation

It is usually stated that apoptosis does not induce an inflammatory response, whereas ischemic cell death does. This needs to be qualified. Once the macrophages have made contact with their apoptotic target, they stick to it by means of vitronectin receptors,[66] but how do they find their target in the first place? They must have been somehow attracted to it, albeit over a short distance, and this sequence is certainly typical of inflammation.

It is true that apoptotic cells do not seem to attract neutrophils or lymphocytes. This could reflect a qualitative difference of apoptotic cell death, but it could also mean that cells dying singly (as apoptotic cells usually do) release such small quantities of chemoattractants that not all the molecular species reach the vascular endothelium in effective concentrations (the endothelial cells are responsible for initiating the sequence of leukocyte emigration; reviewed in reference 15).

When apoptosis occurs on a large scale, as in certain phases of embryonic development, hordes of phagocytes appear on the scene. Saunders and collaborators[67] have beautifully illustrated this phenomenon in situations that they refer to as cataclysmic necrosis, such as the death of cells in the interdigital zones[67–69] (Figure 7). In these situations the entire mass of dead apoptotic cells is replaced by a crowd of mononuclear phagocytes; as matter of fact, the best method for demonstrating this cataclysmic event is to stain the tissue *in vivo* with Nile red, a lysosomotropic dye (reviewed in Reference 15). The dye reveals not the dead cells but the lysosomes of phagocytes in which their debris are packed. The phagocytes are so numerous that, once stained, the clusters can be seen with the naked eye (Figure 7). Microscopically the image is undoubtedly suggestive of inflammation, but the nature of the phagocytic cells is not certain. They are probably not derived from the blood because circulating monocytes are not present[70] at the stages under consideration. They are either tissue macrophages or parenchymal cells ("*Schwesterzellen*"[27]) that became phagocytic. This raises the interesting possibility that phagocytosis by nonprofessional phagocytic cells would be a useful adaptation to programmed cell death in the embryo, when a clean-up operation is required but a full-blown inflammatory response is not yet available.

One inflammatory feature of apoptosis does seem unusual, namely, the fact that the cell debris (apoptotic bodies) are often phagocytized by neighboring cells such as epithelial cells, which are not professional phagocytes. This does appear to be cellular cannibalism, but is it specific to apoptosis, or does it represent a general tendency of cells to devour their

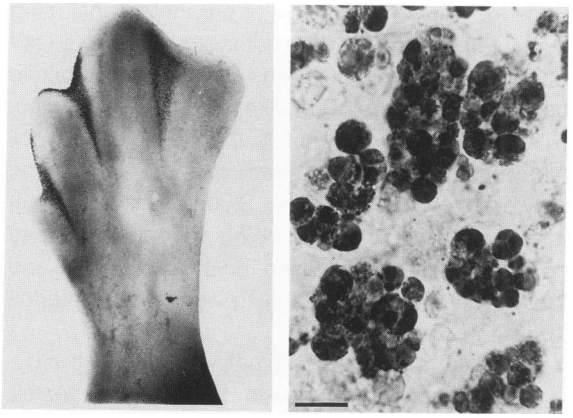

Figure 7. Left: *A classic picture of programmed cell death in the leg bud of a chick embryo, stained* in vivo *with Nile red (which becomes concentrated in phagosomes). The dark interdigital zones represent myriads of red-stained macrophages scavenging the debris of cells that died on schedule.* **Right:** *A squash preparation of a 4-day chick embryo, stained with Nile red, showing phagocytes loaded with debris of dead cells. Bar = 10 μ. (From Saunders and Fallon[66] with permission).*

disabled neighbors, however they may have died? More data are needed.

Apoptosis and Programmed Cell Death

A misunderstanding has arisen here, due to the fact that two different programs are involved in apoptosis: 1) a program to carry out suicide and 2) another program to trigger the suicidal program.

The phenomenon properly called programmed cell death received its name before apoptosis[67,71]; it referred to situations in which cells are programmed to die at a fixed time. Such is the death on schedule of certain clusters of cells in the embryo.[31] For example, in the chick embryonic plate, a group of cells has to die at a precise time to help create the outline of a wing, much as a sculptor hammers off chips of marble to produce a statue. These doomed cells die on schedule even if they are transplanted elsewhere in the embryo.[67] This form of cell death is programmed in the sense that a genetic clock selects a given time for the death of certain cells. When the time has come, a different program must dictate to these cells how to engineer suicide (eg, apoptosis). In most cases the morphology of this death on schedule turns out to be apoptosis, but in other cases it is not, most notably in spermatocytes and spermatids in the course of normal spermatogenesis,[72] in the massive programmed cell death that occurs during the development of the nervous system,[73] or in the massive death of whole organs during the metamorphosis of certain insects.[44] In other words, there are many situations in which programmed cell death occurs by a morphological and biochemical mechanism that is not apoptosis. There is room here for additional discoveries.

In recent years there has been an unfortunate tendency to use programmed cell death and apoptosis interchangeably, because in both cases genetic programs are involved. This is confusing.[74,75] The genetic program of programmed cell death is a clock specifying the time for suicide, whereas the genetic program of apoptosis specifies the weapons (the means) to produce instant suicide.

We therefore recommend that the name programmed cell death continue to be used, as originally proposed, for death on schedule and not as a synonym of apoptosis.

Cell Death Not by Apoptosis: Accidental Cell Death

The discovery, and naming, of apoptosis oblige us to find suitable names for the types of cell death that occur by other modalities. This brings out the problem that the major sore spot in the nomenclature of cell death is precisely the lack of a suitable name for cell death that occurs not by apoptosis but by some external agent. Intuitively, the concept seems simple, as we are referring to cell death by accidental causes, such as heat, which would produce the cellular equivalent of murder. Indeed death by murder has been suggested, half in jest,[76] but it is quite misleading, as cell murder by killer cells, as we have seen, produces apoptosis.[52] Accidental cell death was proposed by Bessis,[59] and it is certainly the best available term, although it is not perfect because accidental causes, such as mild heat or toxic agents, can also induce apoptosis. Necrosis is the term currently used for nonapoptotic, accidental cell death.[77] We find it utterly confusing, because necrosis should not be used to define a mode of death, as we will now explain.

What is Necrosis?

The starting point for answering this question should be that, once again, cell death and necrosis are two very different things. Cell death is a process that leads to the point of no return, which, for liver cells submitted to total ischemia, lies, as stated above, at approximately 150 minutes,[15,32] at which time scarcely any changes can be seen in histological sections. Necrosis is full-blown only after 12 to 24 hours. In other words, cells die long before any necrotic changes can be seen by light microscopy. To say cell death by necrosis implies that the cell dies when it becomes necrotic, which is patently untrue. It is rather like saying that clinical death occurs by postmortem autolysis. Furthermore, necrosis has been used for a very long time (approximately 2000 years) to mean drastic tissue changes visible to the naked eye and therefore occurring well after cell death. It is important, both conceptually and didactically, to preserve this usage.

Necrosis is signaled by irreversible changes in the nucleus (karyolysis, pyknosis, and karyorhexis) and in the cytoplasm (condensation and intense eosinophilia, loss of structure, and fragmentation). We can safely assume that these are the features of a cell's cadaver, whatever the mechanism of the cell's death, be it ischemia, heat, toxins, mechanical trauma, or even apoptosis. The most common microscopic settings of necrosis are 1), cells that died singly displaying the morphological changes of apoptosis, for which we have suggested the term apoptotic necrosis, and 2), groups of cells that died of ischemia, which we can call ischemic necrosis or massive necrosis when the mechanism is not known.

But what was there before ischemic necrosis? Obviously, ischemic cell death. This is the only variety of cell death (other than apoptosis) that has been studied in detail. The data at hand are enough to offer a coherent picture of cell death by ischemia, outlining an entity that can be set up as a counterpart to apoptosis. Because entities need a name, we propose oncosis.

Apoptosis versus *oncosis*

Ischemic cell death is characterized by swelling; thus it should be defined by a name that refers to swelling. There is such a name in the literature, namely, oncosis (from *ónkos*, meaning swelling). This term was coined by von Recklinghausen[78] almost 100 years ago, precisely with the meaning of cell death with swelling. In a monograph on rickets and osteomalacia, published posthumously in 1910, von Recklinghausen described death with swelling primarily in bone cells. It is an obvious but little known fact that osteocytes often die with enlargement of their lacunae and sometimes also of their canaliculi. Pathologists with expertise in bone diseases are familiar with such images, especially in bone tissue that dies by slow ischemia, eg, in the stumps of a fracture (even Virchow illustrates this phenomenon, without giving it a name, in Figure 129 of his Cellular Pathology[2]). Von Recklinghausen's color illustrations of oncotic osteocytes are striking (Figure 8). It is certainly a tour de force for the swelling osteocyte to enlarge its stony lacuna. Von Recklinghausen was probably correct in assuming that this process required an enzymatic effect, which he called trypsis. (Note that Von Recklinghausen included in oncosis also the mode of death of the so-called hypertrophic cells of the growth cartilage. There is no doubt that these cells swell and become hydropic before they die. However, their mode of death, in our view, is still a mystery. Their swelling is probably quite unrelated to hypertrophy, as both Virchow[2] and von Recklinghausen[78] observed. Their mode of death is certainly programmed, which fits with apoptosis, but the swelling does not. Perhaps we are dealing here with yet another form of cell death.)

After von Recklinghausen the term oncosis continued to be used by European pathologists working on bone tissue. We propose that it be given a more general mission, as a counterpart, to apoptosis. It is concise and descriptive. By its reference to swelling it is particularly well suited to contrast with shrinkage necrosis, and it allows necrosis to cover, as it always did, those changes that occur after cell death. In today's medical jargon, the root onco- is not limited to the swelling of tumors (for example, oncotic pressure). Our proposal is summarized in Figure 9. It will be noticed in this figure that necrosis can occur after

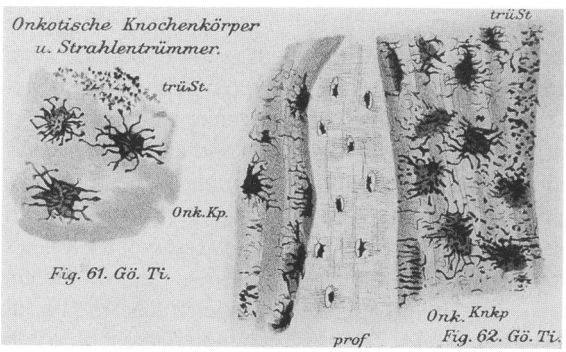

Figure 8. *Oncosis in osteocytes as illustrated by von Recklinghausen in 1910. (reference 78 Tal. XXI). From the tibia of a 30-month-old girl with osteoporotic osteomalacia (rickets). prof, bone lamellae with normal osteocytes; Onk.Kp. and Onk.Knkp., swollen, dying, or dead osteocytes in enlarged lacunae; truSt., cross sections of dilated bone canaliculi. (Sections of undecalcified bone stained with thionine; dilated canaliculi and some lacunae appear black because they are filled with air injected into the bone (reference 78 p 135), a method not known in our day. Original drawing watercolored.)*

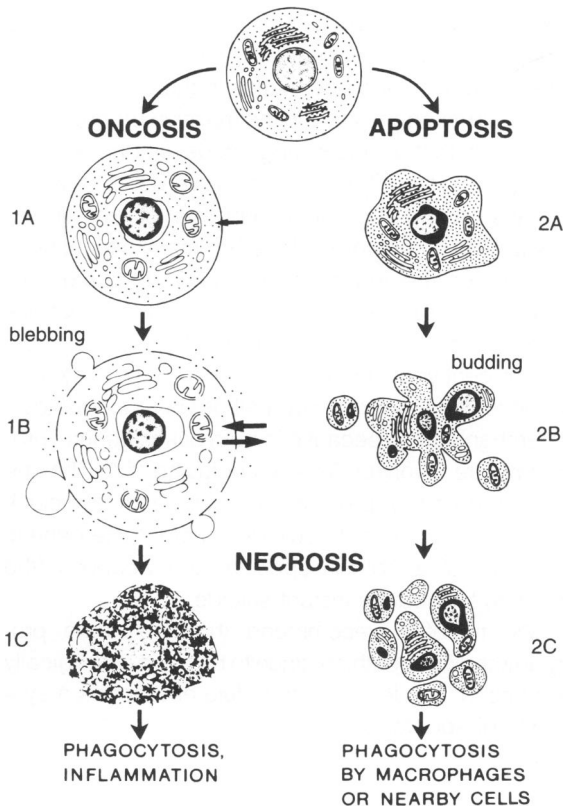

Figure 9. *Two pathways of cell death leading to necrosis. At the top is shown a normal cell.* **1A** *Swelling.* **1B:** *Vacuolization, blebbing, and increased permeability.* **1C:** *Necrotic changes, ie, coagulation, shrinkage, and karyolysis.* **2A:** *Shrinkage and pyknosis.* **2B:** *Budding and karyorhexis.* **2C:** *Necrotic changes, ie, breakup into a cluster of apoptotic bodies (2C adapted from Weedon et al[53]).*

both forms of cell death. A fine example of post-apoptotic necrosis is the cataclysmic necrosis in embryo tissues described by Saunders and Fallon.[66] Kerr and Harmon[50] have also pointed out that apoptotic cell bodies can incur extracellular breakdown and referred to this post-apoptotic change as secondary necrosis, thereby concurring with our view that necrosis can occur also after apoptosis.

In summary, we can define oncosis as follows: 1), oncosis is a form of cell death accompanied by cellular swelling, organelle swelling, blebbing, and increased membrane permeability; 2), its mechanism is based on failure of the ionic pumps of the plasma membrane; 3), it is caused, typically, by ischemia and possibly by toxic agents that interfere with ATP generation or increase the permeability of the plasma membrane; 4), it evolves within 24 hours to typical necrosis; 5), it is usually accompanied by karyolysis; 6), it can be diagnosed by tests of permeability on whole cells, either in suspension (by dye exclusion tests) or by electron microscopy (using a colloidal marker)[79]; 7), the DNA breaks down in a nonspecific fashion[42]; and 8), the cellular changes (increased permeability of the plasma membrane, cell swelling, organelle swelling and vacuolization, and simultaneous protein denaturation and hydrolysis) can only be hinted at by ordinary histological techniques.

Many experiments have shown that blebbing, described above, begins during the early stages of ischemic damage and is initially reversible. Large blebs may burst, and it has been suggested that this may be the final blow to a dying cell (reviewed in reference 15).

Why karyolysis should follow oncosis is not known. As regards the mechanism of karyolysis, a huge literature appeared early in the 1900s, when autolysis was a fashionable research topic (reviewed in references 13, 16, and 48). The basic problem was already identified by Weigert: has the chromatin leaked out, or has it lost its stainability? From the studies of Trump et al[80] it appears that both mechanisms can operate.

In closing, we would like to point out two facts. First, oncosis and apoptosis are merely two forms of cell death, among many others that remain to be described. Consider, for example, that form of cell death that makes histopathology possible, namely death by fixation. Histological fixatives are designed to produce the perfect crime, death without visible traces. What shall we call it? Second, in these days of molecular pathology, it is well to remember that the marvelous story of apoptosis was initiated by a very simple, almost elementary morphological observation, accessible to the microscopes of our great-grandfathers.

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