Apoptosis: a gene-directed programme of cell death

ABSTRACT—Apoptosis is a particular type of programmed cell death which commonly occurs in the developing embryo, in normal healthy adult tissues and in many pathological settings. In contrast to necrosis, apoptosis is not a passive phenomenon but is gene-directed, usually requiring ongoing protein synthesis. The dying cell is characterised by having a raised level of cytosolic Ca^{2+}; this activates a non-lysosomal Ca^{2+}- and Mg^{2+}- dependent endonuclease which digests the chromatin into oligonucleosome length fragments. The dying cell may or may not fragment into a number of apoptotic bodies, but in all cases the cell contents are bounded by a membrane which prevents the spillage of harmful substances such as DNA. Apoptotic cells are eliminated through phagocytosis by neighbouring cells and macrophages, and cell surface changes on apoptotic cells aid their recognition and engulfment by the phagocytosing cells. Extrinsic signals can both stimulate and inhibit apoptosis, and even direct damage to the cell can activate the process. Apoptosis is widely involved in organ formation in the embryo, and its occurrence in response to noxious stimuli such as cytotoxic drugs, irradiation and hyperthermia may be viewed as an altruistic suicide. Apoptosis provides a safe disposal mechanism for neutrophils at inflamed sites, and within the immune system it is considered responsible for eliminating self-reactive T-cell clones and for the affinity maturation of antibody producing cells. A failure to undergo apoptosis has been invoked in the pathogenesis of low-grade follicular lymphoma, and the triggering of apoptosis with monoclonal antibodies specifically in tumour cells has been achieved in one or two cases.

Why do we bother to classify cell death?

Even in these times of rapid developments in our understanding of the molecular processes of disease states, histopathology and its quintessentially observational nature still often leads to the recognition of new phenomena. Thus, in 1980, Wyllie and his colleagues in Edinburgh [1] were able formally to propose a new classification of cell death based on morphological criteria which separated the degenerative phenomena known as necrosis from a second pattern in which the dying cell underwent a progressive contraction of cellular volume, widespread chromatin condensation, but preservation of cytoplasmic organelles. The affected cells then fragmented into a number of membrane-bound bodies which were rapidly phagocytosed by neighbouring cells. This latter type of cell death was termed apoptosis (apo'-pto'-sis: Gr. 'dropping off', as leaves from trees) [2], since it was initially observed in a variety of normal physiological states where it appeared to complement mitosis in maintaining tissue size. Apoptotic cell death is not new to surgical pathological practice, but terms such as necrobiosis, shrinkage necrosis or Councilman bodies (in the case of liver) were used to describe the process. The term 'programmed cell death' is often used synonymously with apoptosis, and while apoptosis is programmed in that it is genetically controlled and influenced by extrinsic and intrinsic signals, there are instances where programmed cell death is not apoptosis. For example, the endometrial lining of the uterus is programmed to undergo ischaemic necrosis at the end of the menstrual cycle, while terminal differentiation in renewing tissues is associated with imminent cell death which also is not by apoptosis. However, cell death during terminal differentiation can have elements of the apoptotic programme such as the enhanced expression of transglutaminase seen in maturing keratinocytes.

Cell death is a common enough occurrence in both healthy and diseased tissues. In the embryo, genetically controlled cell death occurs at precise developmental stages in the process of organogenesis; in the adult, cell death (or loss) must keep pace with cell production in the great renewal systems (bone marrow, gastrointestinal tract, skin) if the tissues are not to expand. Cell death is also a prominent feature of lymph node germinal centres, and is responsible for the collapse of the endometrium at the end of the menstrual cycle. In tumours, cell death and cell loss are major determinants of the rate at which tumours grow, while cell death is the end-result of the unwanted toxicity of many compounds such as paracetamol in the liver, and many anti-cancer cytotoxic drugs which target the proliferating cells in the continually renewing cell populations. If the mechanism of cell death was the same in all these cases and all dead cells underwent the process of necrosis (defined as the sum of the morphological changes which occur *after* death), an essentially passive phenomenon, then a less than enthusiastic approach to the topic would be read-

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ily understandable. However, apoptotic cell death is clearly implicated in many of these instances and, unlike simple degeneration, death depends upon the participation of cellular components which may be activated or suppressed by extrinsic and intrinsic signals. Thus there is the potential for therapeutic manipulation in specifically triggering apoptosis in cancer cells, and over the next few years this will undoubtedly be a major goal of investigators in this field.

Before describing the mechanisms involved in apoptotic cell death it is worth reviewing the perturbations and intracellular events which lead to necrosis, since both necrosis and apoptosis can occur simultaneously in tissues, particularly tumours. Generally speaking, necrosis follows from severe environmental trauma which either directly damages the plasma membrane (complement-induced cytolysis) or interferes with the generation of energy by blocking the synthesis of ATP (anoxia, ischaemia). As a consequence, energy-dependent ion-pumping mechanisms are impaired, causing various ions to move down their concentration gradients across the plasma membrane: notably an entry of sodium and calcium and a loss of potassium. These ion movements result in a loss of volume control by the plasma membrane, causing an influx of water into the cell (cell and organelle oedema, formerly called hydropic degeneration), manifest as acute cell swelling or 'cell ballooning' (Fig. 1). Initially such changes are reversible, but prevailing adverse conditions send the cell on a downward spiral particularly with the sustained increased levels of cytosolic calcium disrupting the cytoskeleton and activating membrane-located degradative phospholipases and proteases. Together with a switch to anaerobic glycolysis, a decrease in intracellular pH, and a reduction of macromolecular synthesis, the affected cell dies, accompanied by the rupture of organelles and the plasma membrane, manifest by light microscopy as coagulative necrosis. In contradistinction, apoptosis is an energy-dependent process, usually requiring macromolecular synthesis which results initially in cell shrinkage rather than cell swelling.

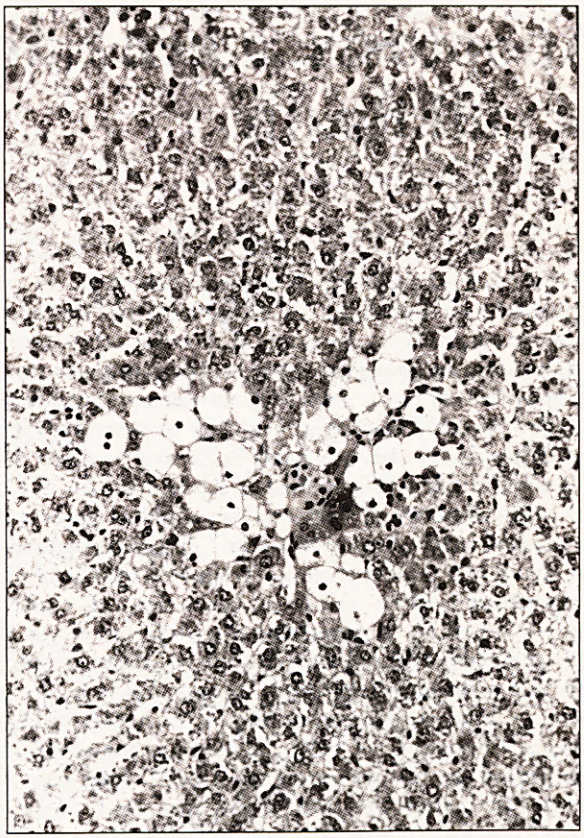


Fig. 1. *A forerunner of necrosis; gross cell oedema in a group of hepatocytes.*

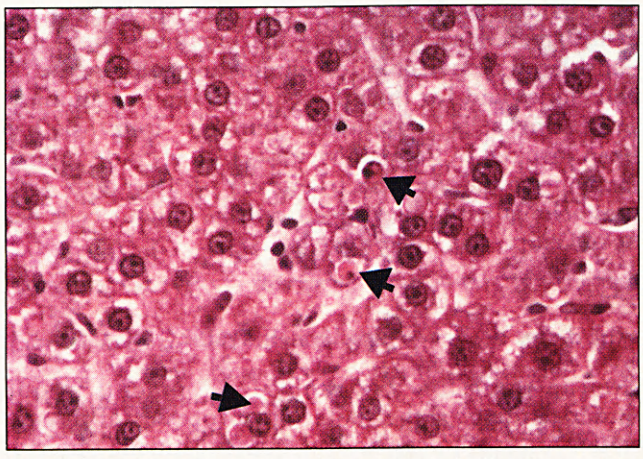


Fig. 2. *At light microscope level, apoptotic bodies (arrows) in the liver.*

Morphology of apoptosis

Light microscopy. Apoptosis is a common process of cell death in a wide variety of tissues where cell deletion needs to occur while maintaining the broad tissue architecture. At light microscope level, apoptotic cells are easily recognisable; they occur individually or in very small groups surrounded by normal healthy tissue. They have condensed, basophilic chromatin, a small amount of eosinophilic cytoplasm, and are typically surrounded by a 'halo' (Fig. 2); larger apoptotic cells break up into fragments, apoptotic bodies, which may or may not have nuclear components, but smaller cells, such as apoptotic thymocytes, do not usually fragment. At these magnifications it is impossible to distinguish with any certainty whether the halo is a result of retraction of the apoptotic cell or body from its neighbours or, as is more likely, a phagocytic vacuole whose cytoplasmic boundaries are too fine to be observed. Apoptotic bodies are rapidly phagocytosed by macrophages or neighbouring epithelial cells (neutrophils play no part), and it seems that the vast major-

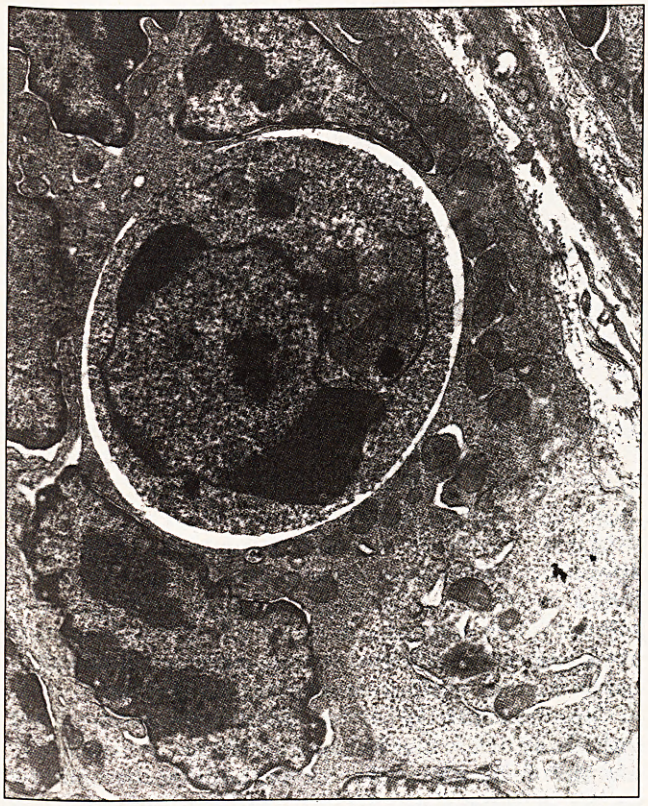


Fig. 3. *Electron micrograph of an early apoptotic cell, illustrating condensation and margination of chromatin to form dense masses that abut on the nuclear membrane; there is also convolution of the nuclear outline.*

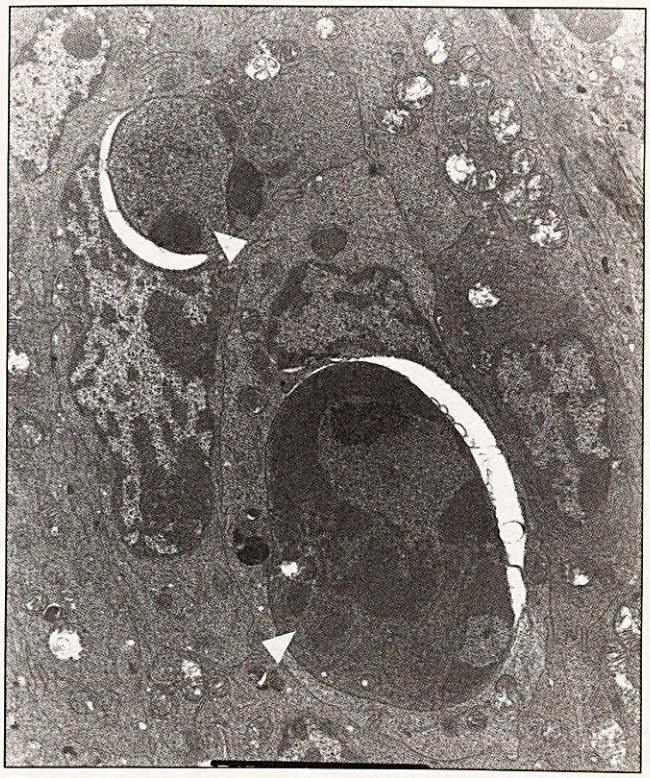


Fig. 4. *Electron micrograph illustrating two apoptotic bodies (arrows) phagocytosed by adjacent intestinal crypt epithelial cells. Note the higher electron density of the apoptotic bodies, the well preserved organelles (endoplasmic reticulum, mitochondria) within them, and how they can distort the nucleus of the ingesting cell.*

ity are already within phagocytosing cells when they become apparent. Where discernible, the only clue to previous phagocytosis of an apoptotic body may be the distortion of the nucleus of the ingesting cell.

Electron microscopy. When ultrastructural features are examined, apoptotic cells are found to have a remarkably stereotyped morphology, no matter in which tissue they are formed or in response to which stimulus. Initially they lose plasma membrane features such as junctions and microvilli and present a smooth outline. Whether free or within a phagocytic vacuole, the earliest intracellular changes are condensation of the cytoplasm and pushing to the periphery of the nucleus one or more dense crescents of chromatin which obscure nuclear envelope pores. At this stage the nuclear membrane frequently blebs (Fig. 3), the predecessor of nuclear fragmentation; the nucleolus progressively disintegrates into osmiophilic granules. The increased electron density of the cytoplasm is a result of cramming together of organelles and free ribosomes (Fig. 4) owing to the reduction in water content of the apoptotic body. Integrity of organelles, however, is largely maintained and there is no initial swelling of the cisternae of endoplasmic reticulum. Mitochondria have none of the distension that is characteristic of necrotic cells and thus remain functional well into late stages of the apoptotic process. It is not unusual for the larger organelles to be sequestered into one or more areas of the cytoplasm so, when fragmentation takes place, organelles are not equally distributed between apoptotic bodies; rather, some are 'organelle heavy' while others contain no more than closely packed ribosomes. The final stages of apoptotic disintegration within phagosomes are similar to degradation of any cellular material within secondary lysosomes with enclosed elements of endoplasmic reticulum finally becoming distended; apoptotic remnants may only indisputably be recognised by the presence of chromatin (Fig. 5). Even at late stages, mitochondria tend to retain their membrane integrity. The major morphological changes associated with apoptosis are summarised in Fig. 6.

The biochemistry of apoptosis with particular reference to endonuclease activation: a final common pathway

Irrespective of whether apoptosis is precipitated by the removal of a normally trophic stimulus, such as the

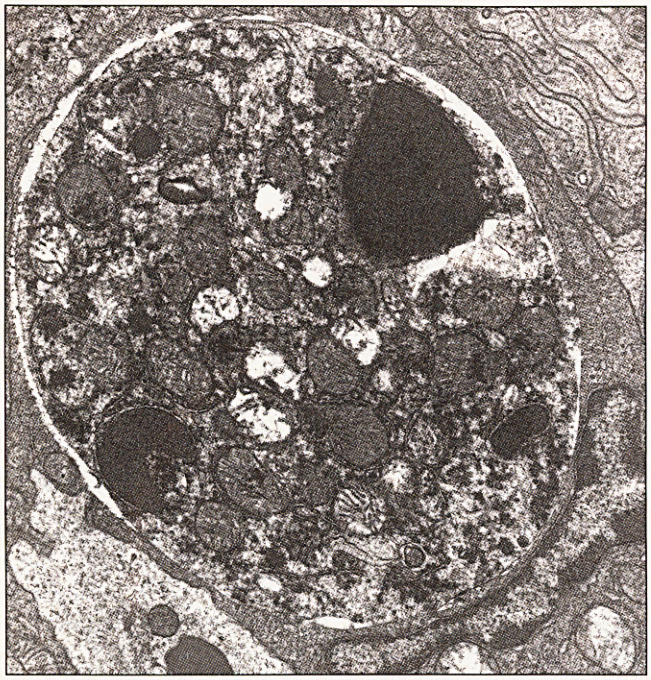


Fig. 5. *Electron micrograph of a large apoptotic body within a crypt epithelial cell; three chromatin fragments are still clearly discernible and there are the beginnings of organelle breakdown.*

absence of testosterone from the prostate gland, or by the addition of a stimulus such as glucocorticoid hormones for immature thymocytes, or even by simply disrupting cell growth with anticancer cytotoxic drugs, the net result is that DNA is always cleaved in a very specific manner which appears responsible for the characteristic nuclear morphological changes. In *in vivo* situations, apoptotic cells are widely dispersed amongst viable cells, making biochemical analysis difficult. However, immature thymocytes from the rat thymus exposed *in vitro* to glucocorticoids have provided a perfect model system for the harvest of large numbers of apoptotic cells. Such cells can easily be separated from 'contaminating' healthy cells since they lose up to one-third of their volume during the process and are therefore much denser. Using such systems it has become clear that the final common pathway leading to the characteristic condensation of the chromatin and to cell death is the cleavage of double-stranded DNA, so resulting in the production of oligonucleosome length fragments of DNA [1, 3, 4].

In eukaryotic cells the DNA double helix in each chromosome is folded in a highly ordered fashion; the fundamental packing unit is the nucleosome, a histone octamer consisting of two copies of each of four histones—small proteins with a high proportion of positively charged amino acids [5]. The nucleosome forms a protein core around which the double stranded DNA helix is wrapped twice, and it appears that in apoptotic cells an enzyme, a non-lysosomal endogenous endonuclease, is activated which digests the DNA (linker DNA) between the nucleosome beads; the rest of the DNA is protected from digestion and remains as double stranded DNA fragments associated with one or more nucleosomes. This apparently ubiquitous pattern of DNA cleavage in apoptotic cells has been elucidated by chromatography of the partially degraded chromatin. If separated by velocity sedimentation on sucrose gradients, the sedimentation pattern indicates particles with stepwise increments in DNA and protein; there is no slowly sedimenting material indicative of deproteinised DNA. Alternatively, chromatin from apoptotic cells can be deproteinised and subjected to agarose gel (a polysaccharide isolated from seaweed) electrophoresis which separates double stranded DNA molecules of different sizes. The negatively charged molecules migrate towards the positive electrode, the smallest molecules migrating furthest; DNA from apoptotic cells always separates with stepwise increments in DNA conforming to integer multiples of a subunit which corresponds to the number of base pairs associated with each nucleosome—hence the so-called ladder pattern of DNA cleavage products (Fig. 7).

Quite recently [6] it has been demonstrated not only that DNA is cleaved specifically at internucleosomal sites in apoptotic thymocytes but that the endonuclease preferentially digests transcriptionally active DNA to small fragments (short oligonucleosomes and mononucleosomes). Inactive heterochromatin is left as relatively long oligonucleosomes, presumably because this chromatin is further packaged as a solenoid containing six nucleosomes per turn which is relatively inaccessible to the endonuclease.

The process of DNA fragmentation is extremely rapid; in the cytolysis of target cells by cytotoxic T lymphocytes (CTL), cleavage of DNA begins within 10 minutes of contact [7]. Another common though not universal characteristic of apoptotic cells is that *de novo* protein synthesis is involved in the process. Using inhibitors of RNA and protein synthesis such as actinomycin D, cycloheximide and emetine, it has been found that some immature thymocytes exposed to glucocorticoids require macromolecular synthesis if DNA fragmentation is to occur [3, 4]. On the other hand, CTL-mediated cell killing does not appear to require protein synthesis [7]. A more general feature of apoptotic cells is that the DNA fragmentation and cell killing are dependent upon a sustained increase in cytosolic Ca^{2+} concentration; for example, calcium ionophore A23187 induces thymocyte apoptosis *in vitro* [3]. Simply incubating freshly isolated thymocytes with Ca^{2+} and Mg^{2+} causes most of the DNA to be fragmented within 90 minutes [4], while blockers of calcium influx into the cell substantially delay apoptosis in the rat prostate gland following androgen deprivation [8]. Such studies all point to the fact that Ca^{2+} and Mg^{2+} are involved in activation of the endonuclease, and Cohen and Duke [4] suggested that the requirement

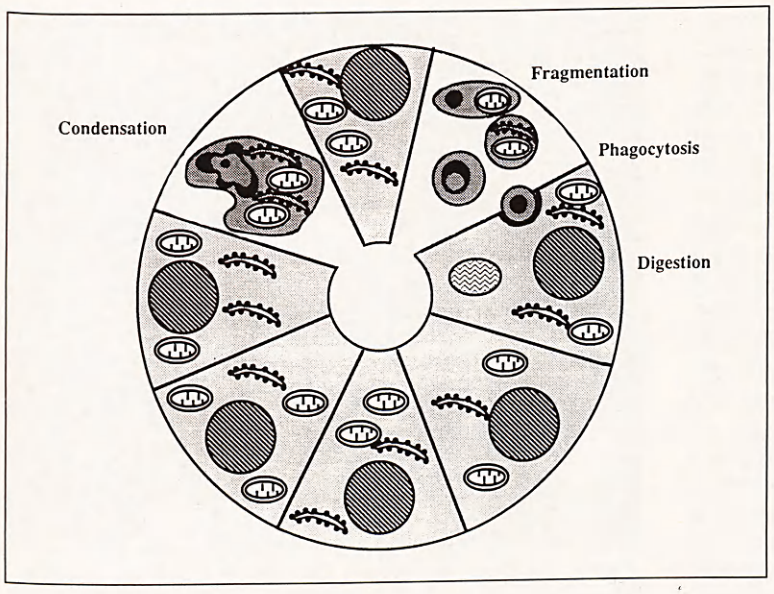


Fig. 6. *Summary diagram of the morphological events in apoptosis in an acinus of a glandular tissue. The earliest stage is characterised by a condensation of the cell, margination of chromatin and convolution of nuclear and cellular outlines. The apoptotic cell soon fragments into a number of apoptotic bodies which are rapidly phagocytosed by neighbouring cells, and digestion is accomplished within heterophagolysosomes.*

for protein synthesis in thymocytes undergoing apoptosis is part of a cytoplasm-to-nucleus calcium transport system. An increase in cytosolic Ca^{2+} induced by A23187 with a concomitant block in endonuclease activity successfully prevents thymocyte DNA degradation [9]; in the same model of ionophore-induced apoptosis, inhibitors of RNA and protein synthesis will block endonuclease activity [10]. These data suggested that a protein with rapid turnover, possibly the endonuclease itself, was required for calcium-dependent DNA fragmentation. The inhibitory effect of compounds like actinomycin D and cycloheximide on DNA fragmentation could be reduced if the thymocytes were pretreated with a combination of proteases that blocked proteolytic breakdown. Since Ca^{2+} alone will support extensive DNA degradation, it was concluded that the endogenous endonuclease was constitutive in immature thymocytes and undergoes rapid turnover, and that agents which block mRNA or protein synthesis inhibit the synthesis of new endonuclease. Protein synthesis is also important for mediating the influx of calcium, and it is assumed, for example in the case of thymocytes, that the interaction of glucocorticoid with its plasma membrane receptor rapidly induces the synthesis of a protein which acts as a calcium pore [11].

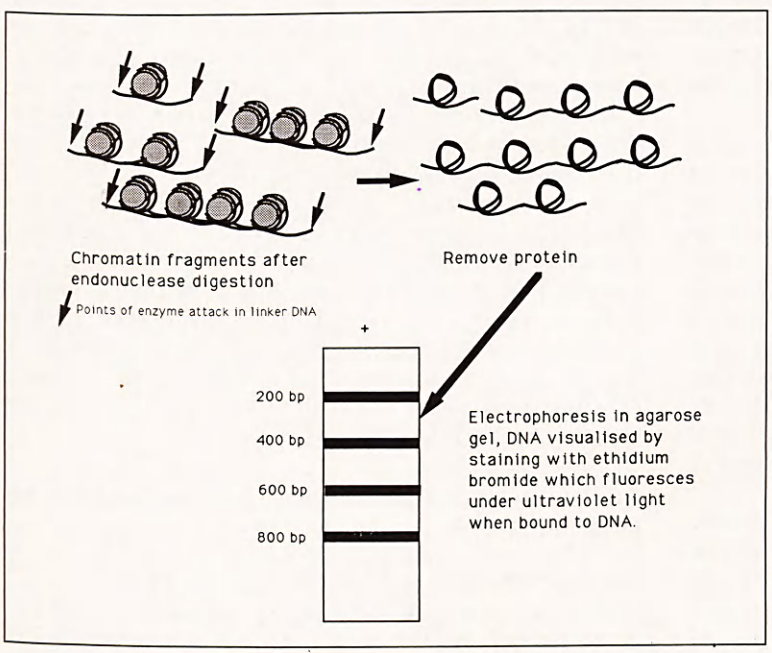


Fig. 7. *Simplified scheme for the electrophoretic analysis of DNA from apoptotic cells; for clarity the largest fragment has only four nucleosomes.*

Transglutaminases are another group of Ca^{2+}-dependent enzymes which appear to be activated during programmed cell death. This family of enzymes is involved in crosslinking proteins to form large protein polymers; for example, keratinocyte transglutaminase is intimately involved in the production of the cornified envelopes during the terminal differentiation of epidermal cells—a type of programmed cell death. Similar increases in tissue transglutaminase have now been found in apoptotic hepatocytes following a period of hyperplasia [12], and it is likely that the enzyme functions to provide a highly crosslinked protein scaffold in apoptotic cells joining cytoplasmic and membrane proteins which maintain cellular integrity during the formation of apoptotic bodies.

In summary, apoptotic cell death ultimately appears to be a stereotyped cellular response involving synthetic activity, which has the effect of activating a Ca^{2+}/Mg^{2+}-dependent non-lysosomal endonuclease. It is quite clear that there is not an indiscriminate proteolytic digestion of chromatin accompanying this activation, but rather nuclear DNA undergoes widespread cleavage at internucleosomal sites; similar enzymes are responsible for cleaving DNA in terminally differentiating lens cells and normoblasts.

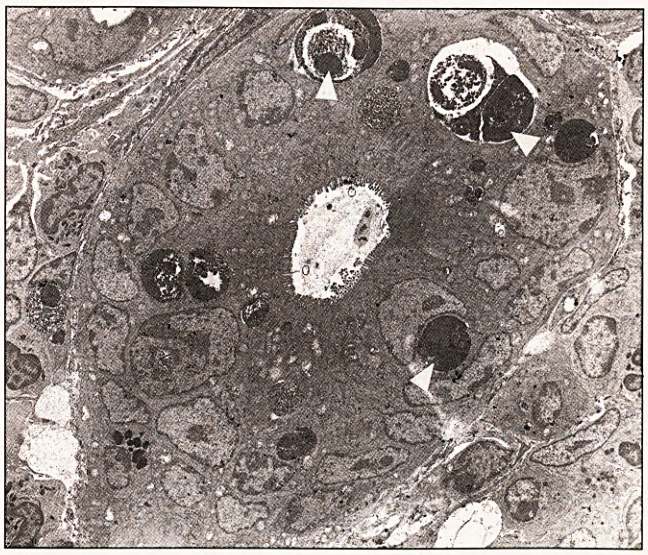


Fig. 8. *Electron micrograph illustrating numerous apoptotic bodies (arrows) within intestinal crypt cells.*

The fate of apoptotic cells

Having undergone fragmentation into a number of membrane-bounded apoptotic bodies, the dead cell is disposed of neatly and efficiently by seemingly specific recognition and phagocytosis by neighbouring cells. Most cells seem capable of phagocytosing apoptotic bodies; in glandular organs the parenchymal cells (Fig. 8) and tissue macrophages seem equally capable, in lymph node germinal centres the 'tingible body' macrophages are testimony to the ingestion of apoptotic B cell progeny, and in tumours even the tumour cells themselves are efficient phagocytosers of apoptotic tumour cells. Once inside the ingesting cell, the apoptotic body, safely packaged in its heterophagic vacuole, undergoes fusion with lysosomes and is rapidly degraded by a process akin to necrosis. Thus, in the case of cell-damaging agents inducing apoptosis, the whole process appears geared to minimising the possibility that damaged or mutated DNA could be transferred from injured cells to healthy cells, so exposing them to harmful genes. Initially the DNA is degraded to non-functional subunits, and then the constituents of the cell are packaged into membrane-bounded fragments to be further concealed by the enveloping membrane of the heterophagic vacuole and heterophagolysosome during their sojourn in the ingesting cell.

If the safe disposal of damaged DNA is one reason for the body invoking apoptosis, then there are probably many others. One such biologically beneficial example appears to be in the removal of neutrophils from inflamed sites [13]. If acute inflammatory lesions are to be resolved and not progress to chronicity, then neutrophils must be effectively disposed of by a manner which prevents the disgorgement of granule contents which would otherwise lead to further tissue injury and a perpetuation of the inflammatory response. Macrophage engulfment of apoptotic neutrophils is the mechanism which provides this safe disposal route, and even neutrophils with a full component of lytic enzymes can suffer such a fate since they appear to undergo time-dependent senescent changes which aid their recognition by macrophages. The recognition of aged neutrophils by macrophages involves the vitronectin receptor (an extracellular matrix receptor of the integrin superfamily) on the surface of the macrophage [14]. In the *in vitro* glucocorticoid-induced model of thymocyte apoptosis, macrophages bind apoptotic thymocytes by a carbohydrate dependent mechanism [15, 16]. Incubating macrophages with monosaccharides such as *N*-acetyl glucosamine prior to the addition of thymocytes blocks macrophage recognition of the steroid-treated cells, suggesting an interaction between a lectin-like macrophage surface receptor and a thymocyte carbohydrate moiety. An overall or local increase in, for example *N*-acetyl glucosamine could be achieved by a loss of the terminal residues (such as *N*-acetyl neuraminic acid) from the cell surface glycans of the apoptotic cells, thus exposing the sugars.

The incidence of apoptosis and the signals for its induction

Large numbers of cells can be deleted from living tissues by apoptosis without significant disturbance of the overall tissue architecture. The process is frequently

found in situations where cell death is physiological, for example in developmental growth, in normal cell turnover and in maintaining homeostasis within the immune system. However, apoptosis is also encountered in many pathological states, be it in cell killing by effector cells of the immune system, in the involution of endocrine target tissues or in tumours. It also occurs in response to injurious stimuli such as hyperthermia and anticancer cytotoxic drugs. Clearly, the stimuli eliciting the same end-result in such a variety of settings will not be the same; there could be intrinsic cellular programmes, but more likely a host of extrinsic signals dependent on the target cell, and in other cases cell injury itself, may be the precipitating event.

Developmental growth

Substantial cell death is part and parcel of normal embryonic development; it even occurs extensively in early human blastocysts in both trophectoderm and the inner cell mass. Apoptosis is deemed responsible for the moulding of many tissues in the embryo, for the coring out of the lumina of many tubes, and for the death of up to 80% of neurones in catering for the needs of the innervated periphery [17]. Cell death in the embryo is certainly 'programmed' in that it occurs at precise developmental stages and is under genetic control, but this should not be taken to imply that all cell death is through the mechanism of apoptosis. In one of the most widely studied systems, that of the developing chick limb bud, extensive cell death occurs in the mesenchymal areas to separate the chondrifying digits; this is usually referred to as necrosis [18]. Within the embryo there are clearly concentration gradients of morphogens (growth factors, retinoic acid etc) which provide the cues to specify fate in embryonic fields, and the relative absence or presence of such factors is likely to be instrumental in causing cell death. There seems little evidence to support the idea that any cell has an intrinsic and thus autonomous control over its own destiny, though the term 'programmed' was originally coined with the apparent connotation of cell suicide. Only in *Caenorhabditis elegans*, a nematode worm with less than 1,000 cells, does cell death appear to be intrinsically programmed in something over 10% of cells, and mutations of cell death (ced) genes appear to promote the survival of cells that would normally die [19].

Cell death by apoptosis is crucial for normal mammalian palate development, and failure of this process is one cause of a cleft palate [20]. At a precise developmental stage the bilateral palatal shelves elevate to a horizontal position above the dorsum of the tongue, the opposing shelves make contact with each other, and the medial edge epithelia adhere to each other to form the midline epithelial seam. It is the failure of this epithelium to undergo apoptosis which can prevent mesenchyme continuity across the palate resulting in a cleft palate. As in other examples of vertebrate development where apoptosis occurs, there does not appear to be wholesale intrinsically controlled epithelial suicide but rather the epithelial cells receive signals (extracellular matrix components, growth factors) from the underlying mesenchyme. Elegant recombination experiments involving epithelium and mesenchyme from different species have clearly highlighted the instructive powers of the mesenchyme; combining mouse palatal epithelium with alligator mesenchyme causes the epithelium to migrate rather than die, the normal behaviour of alligator palatal epithelium. Thus, though palatal medial edge epithelial cell death has been likened to 'murder' by the mesenchyme, it is nevertheless programmed by extrinsic signals resulting in apoptosis.

Normal cell turnover

Careful examination of any histological section will probably identify the occasional apoptotic cell. In the gastrointestinal tract such cells often occur within the proliferative compartment of the crypt, though in all similarly rapidly renewing populations terminal differentiation heralds the death or at least the loss of a cell in the not too distant future by a programmed process which is *not* apoptosis. One suspects that the elimination of isolated cells by apoptosis in normal adult tissues may be an altruistic suicide process to rid the body of potentially harmful cells, eg those with a chromosomal imbalance. In classical endocrine target tissue such as the breast, apoptosis of acinar cells at the end of the menstrual cycle would appear to counterbalance the little cell proliferation that occurs during the first half of the cycle [21].

Within the haemopoietic system, the suppression of apoptotic cell death in precursor cells requires the continued presence of colony stimulating factors such as interleukin-3 [22]. As in other systems, this cell death can be reduced by inhibiting protein synthesis with cycloheximide. The introduction and expression of the proto-oncogene Bcl-2 (B-cell lymphoma) will in fact prolong the survival of these cells after IL-3 withdrawal, and the location of the gene product on the inner surface of the mitochondrial membrane suggests that oxidative phosphorylation and/or electron and metabolite transport are involved in the survival mechanism [23]. Deregulation of Bcl-2 gene expression has been implicated in the pathogenesis of follicular lymphoma (see below).

The immune system

The immune system acquires the ability to discriminate between self and non-self so that destructive autoimmunity is avoided. This self-tolerance may be achieved through the deletion of autoreactive T-cell clones during thymic maturation. This negative selec-

tion process is thought to involve interactions between antigen-specific thymocyte receptors and self-antigen presented by the MHC proteins on accessory cells, thus resulting in the activation of apoptosis in 'forbidden' T-cell clones [24]. This can be demonstrated experimentally through the killing of immature thymocytes by stimulation via the CD3/T cell receptor complex using antibodies, and once again an early sustained rise in cytosolic Ca^{2+} is a prominent feature [25]. The factors that regulate proliferation and death in the thymus are not fully understood, but it seems quite clear that increases in cytosolic Ca^{2+} and an activation of protein kinase C (PKC, an enzyme that adds phosphate groups to serine and threonine amino acids) have important roles in the earliest phases of signal transduction. In thymocytes, a rise in cytosolic Ca^{2+} through antigenic or lectin challenge can lead to proliferation rather than death, and in such circumstances it would appear that the status of PKC activity is crucial—PKC activation protects cells from Ca^{2+} activated endonuclease activation. McConkey *et al* [26] have introduced the concept of 'unbalanced signalling', whereby the same antigen may be stimulatory (proliferation) or inhibitory (death) depending upon the presence or absence of at least one other independent signal, eg interleukin-1 stimulation of PKC. Quite recently two mRNA species unique to dying thymocytes have been identified [27]. One gene product was a membrane protein, while the other protein contained a zinc finger suggesting it might function as a DNA-binding protein regulating transcription. It remains to be seen how ubiquitous such death genes are in other models of apoptosis.

Apoptosis would also appear to be involved in the selection, by immunising antigen, of the B cell progeny (centrocytes) producing antibodies of the highest affinities, with apoptosis of those cells not receiving a sufficiently positive signal from the antigen, ie the cells producing antibody of lowest affinity [28]. This 'affinity maturation' is thought to operate when B cells are activated by antigen to generate memory cells, which with time generates antibodies of increasing affinity against an immunising antigen. This is achieved by the very rapid accumulation of mutations (somatic hypermutation) in the antibody V-region coding sequences after antigenic challenge, and the cells which either fail to modify or actually decrease the affinity of antibody are the ones to be eliminated by apoptosis.

Defects in this selection process have been suggested as being important in the pathogenesis of low grade follicular lymphoma [23]. Such lymphomas have a (14;18) translocation involving apposition of the Bcl-2 gene and the immunoglobulin heavy-chain locus, thus placing Bcl-2 next to a highly transcribed gene. Deregulated Bcl-2 resulting in elevated levels of Bcl-2 mRNA and protein could thus override the apoptotic tendency of germinal centre cells since the Bcl-2 gene product *protects* against apoptosis.

Enhanced B-cell survival, ie protection from apoptosis, has also been suggested as the mechanism by which Epstein–Barr virus (EBV) infected B cells remain long-lived [29]. Using EBV-positive Burkitt's lymphoma cells, it was found that the activation of all eight EBV latent genes was required to protect the cells from undergoing experimentally induced apoptosis. In fact the apoptotic death of tumour cells is a recognised feature of Burkitt's lymphoma, conceivably a phenotypic vestige of the tumour's origin from the germinal centres of lymphoid follicles. Indeed, fewer apoptoses have been found in the neoplastic follicles of follicular lymphomas than in germinal centres.

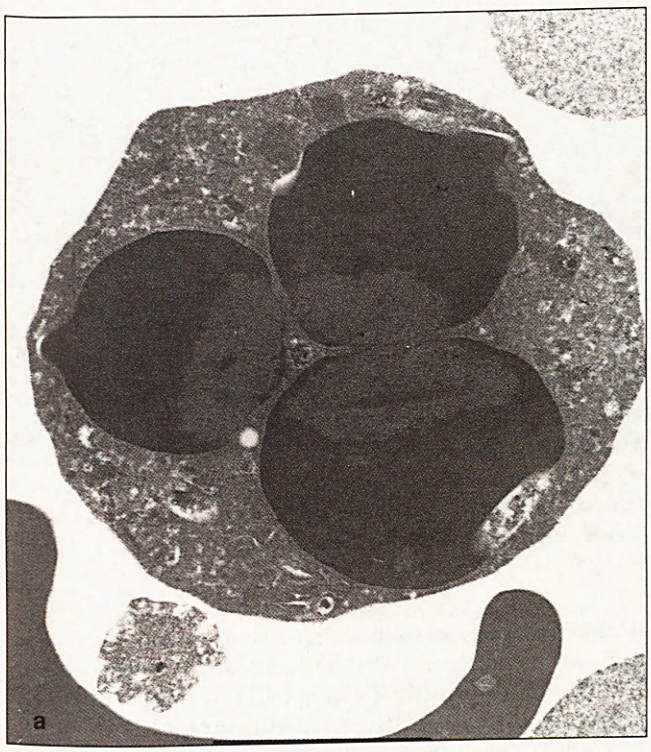
Cell death is an important component of most immunological reactions; unlike the necrosis caused by complement, the effector cells [cytotoxic T lymphocytes (CTLs), natural killer (NK) or killer (K) cells] all cause apoptosis of their targets [16]. All such cell-mediated cytotoxicity probably involves the cell binding to the target, a Ca^{2+}-dependent phase involving secretion of lymphocyte vesicle contents which programmes the cell for death, and ultimately apoptosis. CTLs are involved in many examples of cell killing, including graft rejection, graft versus host disease (GVHD) and all types of viral hepatitis (where apoptotic hepatocytes are often called Councilman bodies or acidophil bodies); NK cells are particularly implicated in the killing of tumour cells and virus infected cells, while K cells are involved in antibody dependent cell-mediated cytotoxicity.

Resolution of inflammation

The efficient removal of neutrophils from inflamed sites once their biological purpose (killing of invading pathogens, breakdown of dead tissue) is complete is a highly desirable objective. Neutrophils can undergo apoptosis, and associated cell surface changes aid their phagocytosis by macrophages [13]. This process probably operates wherever a need to curtail suppurative exudation exists, be it in the resolution of pneumonia (Fig. 9) or the mopping-up of an abscess cavity (Fig.10).

Reversal of hyperplasia

Many glandular epithelia undergo transient hyperplastic reactions in response to overstimulation by a trophic hormone, or the mitogen may be a substrate of cytochrome P_{450} dependent enzymes, as in the case of the liver. Once the stimulus is removed the target tissue returns to its normal size but, rather than allowing 'wear and tear' cell loss in the absence of renewal to cause tissue reduction, most tissues return rapidly to normality through the apoptotic death of the excess cells [30,31]. Interestingly, the production of apoptotic cells can be abruptly halted by re-introduction of the mitogen.



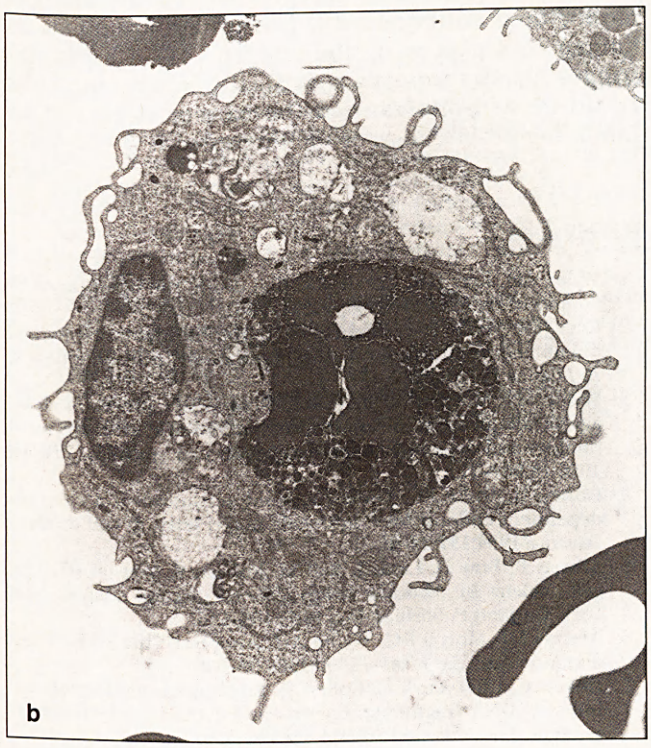


Fig. 9. (a) *Electron micrograph of a human apoptotic neutrophil isolated by bronchial lavage, with characteristic crescents of compacted chromatin in all three lobes.* (b) *Electron micrograph of a pleural macrophage that has engulfed an apoptotic neutrophil which is easily recognisable by its high content of cytoplasmic granules.*

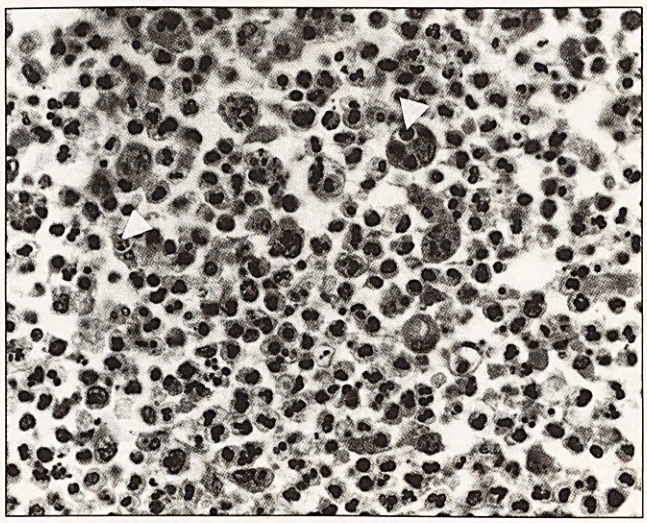


Fig. 10. *Apoptotic neutrophils from an abscess cavity, many of which have been phagocytosed by macrophages (arrows).*

Endocrine target tissue involution

The most widely studied model of such a process is the castration-induced involution of the rat prostate gland. Prostatic acinar cells are dependent upon testicular androgen for their normal function, and castration sets in train a series of regressive changes including atrophy of some cells and apoptosis in others. Exogenously administered testosterone can inhibit castration-induced apoptosis, and it appears that cell death is intimately linked to the expression of the TRPM-2 gene (testosterone-repressed prostate message-2 gene), whose function is yet to be elucidated [32].

Anticancer cytotoxic drugs and hyperthermia

Although apoptosis is perceived by some as exclusively a type of 'physiological' cell death, this is clearly incorrect and apoptosis occurs in response to a wide variety of noxious stimuli [33]. From a teleological point of view it seems much more reasonable to eliminate injured cells by apoptosis than to allow the potentially harmful process of necrosis to occur. It is generally believed that mild forms of injury induce apoptosis while more severe forms of insult result in necrosis. Thus a threshold of injury may exist beyond which necrosis occurs in a given cell, and indeed there is some experimental evidence to support this notion [34]. To date we are largely ignorant as to how a highly conserved process (apoptosis) can be initiated by a widely disparate group of agents (eg antimetabolites, alkylating agents, antitumour antibiotics, cisplatin), but interruption of the carefully integrated series of cell cycle events leading to unbalanced growth could be the stimulus.

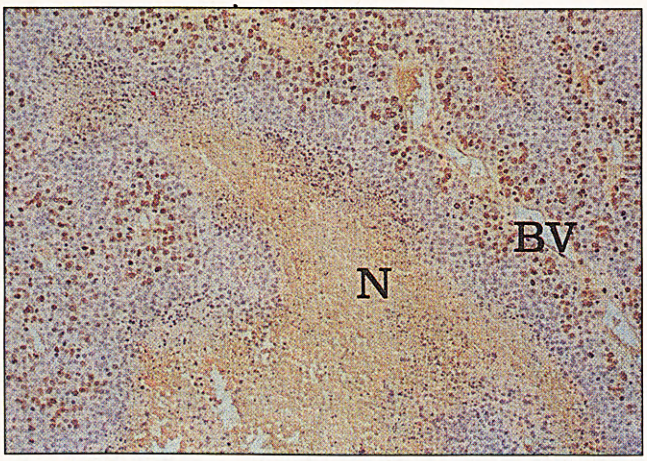


Fig. 11. *In tumours, large areas of cell death (necrosis N) occur in areas most distant from the blood vessels (BV). In contrast, cells undergoing DNA synthesis (labelled here with bromodeoxyuridine and visualised with a peroxidase-conjugated monoclonal antibody and so appearing brown) are found in the most oxic regions.*

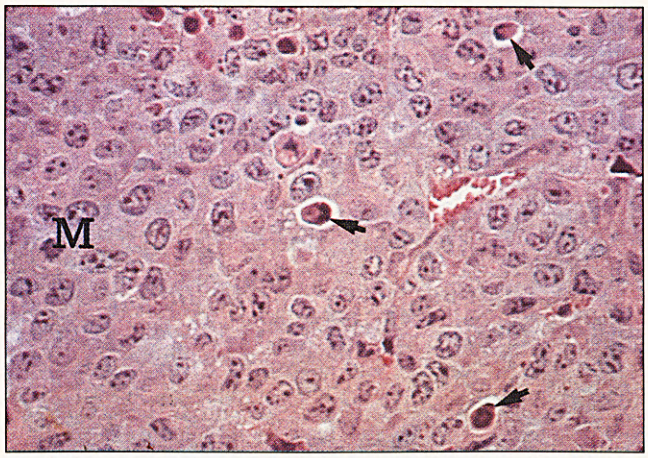


Fig. 12. *In tumours, apoptotic cells (arrows) are usually found singularly amongst viable tumour cells, often close to blood vessels and near mitotic cells (M).*

Neoplasia

It is widely appreciated that in most experimental and human tumours there is a gross disparity between the *observed* rate of growth of the tumour population and the rate of growth we would expect from a consideration of the rate of cell production in that population [35,36]. The difference is due to cell loss from the tumour, most of which is accounted for by the familiar 'lakes' of necrosis in areas most disadvantaged with respect to the afferent blood supply (Fig. 11), and in most, if not all, solid tumours an increasing rate of cell loss rather than a diminished rate of cell production is largely responsible for the curtailment of growth manifest by the typical sigmoid growth curve [37]. However, apoptosis also occurs widely in tumours, the frequency of which is not related to the proximity of the vasculature, and it is not uncommon to find apoptotic tumour cells next to dividing cells (Fig. 12). Why tumour cells undergo apoptosis is not clear, but there are several possibilities: a chromosomal imbalance, a lack of growth factors or the activities of CTLs, NK- or K-cells could be responsible. Tumour necrosis factor (TNF) secreted by macrophages can also stimulate apoptosis [38], and there is also the intriguing possibility that apoptosis represents a residual attempt at autoregulation which ultimately fails, since in some tumours apoptosis is more common when they are small [39].

As already mentioned, overexpression of the Bcl-2 gene has been implicated in the pathogenesis of follicular B cell lymphomas by protecting cells from apoptosis. A most attractive idea therefore is to stimulate apoptosis in tumour cells, and some progress is being made in this area. In human T-lymphotropic virus type 1 (HTLV-1) associated malignant disorders there is a cell surface antigen (APO-1), a 52 kd protein, to which a monoclonal antibody (anti-APO-1) can bind and cause apoptosis [40]. Another surface antigen is known as Fas, a plasma membrane spanning polypeptide related to the TNF receptor family, and anti-Fas monoclonal antibodies will precipitate apoptosis in human cells expressing the antigen [41]. Clearly the triggering of apoptosis specifically in tumour cells could be as important to the cancer therapist as inhibiting proliferation or limiting spread.

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