Death in normal and neoplastic cells

A. H. WYLLIE

From the Department of Pathology, University of Edinburgh

It is well known that cell loss occurs from growing neoplasms *in vivo*. This is particularly prominent in slowly growing tumours, including many human examples (Steel, 1967), and in some cases the rate of cell loss may be as much as 80% of the rate of cell production (Terz, Curutchet, and Lawrence, 1971). Whilst metastasis and desquamation may account for some of the observed cell loss much of it must be due to death of tumour cells *in situ*. Cell death on this scale, sufficient materially to influence the overall growth rate, has for years provoked enquiry but little is known of its mechanism. It is often regarded as a feature of abnormality, either in the environment within the tumour or in the neoplastic cells themselves, but the claim has also been made that it represents the retention by tumour cells of a basic physiological function concerned also with the control of normal cell populations (Laird, 1969). The problem is of more than academic interest, since relatively minor increases in the cell death rate, could they be induced without corresponding increases in cell production, would ensure regression of many human tumours. In an attempt to clarify some aspects of this problem, this paper reviews the phenomenon of cell death in both normal and neoplastic populations.

Cell Death amongst Non-neoplastic Cells

Two concepts of cell death have grown up side by side. The first regards the dying cell as the innocent and probably passive victim of environmental perturbation and is supported by the results of many experiments in which living cells have been exposed to lethal conditions (David, 1965; McLean, McLean, and Judah, 1965). The second regards death as an active intrinsically programmed event, and until recently has been supported almost entirely by the observations of embryologists, who have noted that a selective, orderly deletion of cells is essential in the normal development of many organs (Glücksman, 1951). Conventional morphological accounts of cell death frequently mention nuclear changes seen by light microscopy, for example, karyorrhexis and pyknosis. These changes occur in a variety of conditions and do not necessarily reflect the ultra-

structural morphology. Ultrastructural studies, however, reveal two major modes of cell death (Kerr, 1971), the first commonly found amongst cells placed in overtly lethal environments and conforming to the classically described 'coagulative necrosis', whilst the second has been described more often amongst cells whose death might be expected to be a programmed event. This second mode of death has recently been named 'apoptosis' (Kerr, Wyllie, and Currie, 1972), a term derived from the Greek word meaning the falling off, as of leaves from trees, in an attempt to distinguish it from coagulative necrosis and draw attention to its possible role in the regulation of cell populations.

COAGULATIVE NECROSIS

The mode of evolution of coagulative necrosis probably follows the scheme suggested by Trump and Ginn (1969) (fig 1). Whilst minor injury may permit life, the injured cell adapting reversibly to a new steady state of homeostatic activity, major injury is followed by the irreversible loss of homeostatic regulation. This, the theoretical point of death, is followed by a period of necrosis in which cellular constituents reach physicochemical equilibrium with themselves and their environment. This point seems to involve changes in membrane permeability (Saladino and Trump, 1968), in which calcium ion may play a central role (Judah, Ahmed, and McLean, 1964), resulting in the entry of sodium and water into the cell. The altered permeability underlies the observed morphological changes which involve swelling of cytoplasm and its organelles, with ultimate rupture of the plasma membrane, separation of junctional complexes, and fragmentation of the nucleus. There is variation in the sequence and time course of these events depending on the lethal stimulus, and certain stimuli may provoke additional morphological patterns on their own: for example, early rupture of cell membranes and ballooning of cytoplasm are associated with complement-induced damage *in vitro* (Hawkins, Ericsson, Biberfeld, and Trump, 1972). Coagulative necrosis usually involves sheets of cells at once; it is seen following severe hypoxia and exposure to many toxins both *in vitro* and *in vivo*, and occurs in target cells in at least

Fig 1 Scheme of events in coagulative necrosis. Redrawn from Trump and Ginn (1969).

Fig 2 Scheme of events in apoptosis (Kerr, Wyllie, and Currie, 1972).

one type of cell-mediated immune reaction *in vitro* (Weiss, 1968). Perhaps because of leakage of intracellular materials it usually elicits an inflammatory response in adjacent viable tissues (Majno, 1964).

Coagulative necrosis has never been conclusively demonstrated in the course of embryogenesis and there is little evidence that it is ever involved in the controlled regulation of cell populations.

APOPTOSIS

By contrast, what is known of the morphology, time course, incidence, and mechanism of apoptosis suggests that this may be the controlled phenomenon originally invoked by embryologists.

Morphology

Morphologically apoptosis differs in many respects from coagulative necrosis (Kerr *et al*, 1972) (fig 2). Characteristically it involves single cells whose neighbours remain viable and maintain the overall structure of the tissue in question. There is no inflammatory reaction. Apoptotic cells appear in the light microscope as inconspicuous, membrane-bound portions of eosinophilic cytoplasm, smaller than adjacent normal cells and often containing pyknotic nuclear fragments (fig 3, inset). Ultrastructurally, however, a distinctive sequence of events can be

Inset An apoptotic epithelial cell, distinguished from its 'viable' neighbours in the light microscope by its denser cytoplasm, rounded contour, and fragmented nuclear material, lies adjacent to a sinusoid. Haematoxylin and eosin. $\times 1500$ (by courtesy of the Journal of Pathology).

Fig 3 Rat adrenal cortex. A cluster of apoptotic bodies in the perisinusoidal space (P). Organelles, though morphologically intact, are more densely packed together in the apoptotic bodies (arrows) than in the adjacent epithelial cells (E). Note the spherical mitochondria with spherical cristae characteristic of adrenocortical epithelium. Portions of endothelial cells lining the sinusoid are also visible (S), clearly recognizable by their shape and conventional mitochondria. One apoptotic body appears to have been ingested by the sinusoid endothelium (top right) whilst another lies in the lumen (top centre). Uranyl acetate and lead citrate. $\times 14\,700$.

Fig 4 Rat adrenal cortex. Within a histiocyte (H) there lie several apoptotic bodies, containing condensed organelles characteristic of epithelial cells, and including in one case a nuclear fragment with no bounding membrane (N). Whilst in two ingested bodies the organelles are well preserved (see inset) in the third (on the right) advanced degradative changes are evident. A whorled lysosomal residual body is also present within the histiocyte (bottom) whose state of degradation precludes assessment of its origin. Uranyl acetate and lead citrate. $\times 11\,000$. Inset $\times 25\,000$ (by courtesy of the Journal of Pathology).

traced. The earliest changes involve the nucleus whose chromatin marginates and clumps and whose membrane crenates, sometimes with the appearance of broadening of the perinuclear envelope. About the same time, early in the process, the affected cell loses contact with its neighbours, rounding up and then undergoing cytoplasmic condensation, sometimes to a remarkable degree. In the course of this, cytosol volume diminishes so that the affected cell may adopt bizarre convoluted forms and its organelles become packed close together. While the condensation proceeds the nuclear membrane disappears and coarse masses of chromatin lie free in the cytoplasm. This nuclear disruption contrasts with a striking maintenance of structural integrity in the cytoplasmic organelles, despite their compaction. Eventually the convoluted cell breaks up into a number of membrane-bounded bodies—apoptotic bodies—each containing the organelles present in the part of the cell from which it was derived (fig 3). These bodies may vary in size from something similar to, though smaller than, the original living cell, to tiny membrane-bounded droplets discernible only with the electron microscope. The ultimate fate of apoptotic bodies is often ingestion, either by adjacent epithelial cells or by neighbouring phagocytic cells such as histiocytes or endothelial cells (fig 4). Some may leave the organ by the blood stream or in appropriate circumstances down a lumen. Once within the phagosome of the ingesting cell the apoptotic bodies begin to show changes similar to coagulative necrosis: the mitochondria swell, develop flocculent matrix densities, and lose their cristae; there follows progressive disorganization and dissolution of membranes. The ultimate appearance of the phagosome content is similar to any other lysosomal residual body (fig 4). It should be noted that the evidence is against activation of lysosomal enzymes before this phase; they do not appear to be important in the initiation of apoptosis (Kerr, 1972).

Duration

By studying sections of tissues harvested sequentially after a stimulus promoting apoptosis it is possible to gain some idea of the duration of the process. The half-life of disappearance of apoptotic bodies, as seen by the light microscope, is short: in the adrenal cortex no longer than nine hours. Our impressions from comparing thick and adjacent ultra-thin araldite-embedded sections is that the majority of apoptotic bodies observed with the light microscope are already in at least the initial stages of uptake by other cells. The earliest extra-phagocytic phases of apoptosis seem to be effected very swiftly.

Incidence

The morphological mode of cell death we have called apoptosis occurs frequently in ontogenesis, at utterly predictable times of development specifically deleting certain cells. For example it has been fairly extensively studied by embryologists in the fashioning of digits, where it is responsible for removing the interdigital web and in the moulding of limb buds (Saunders, 1966). It is the means of removal of unwanted epithelium during palate fusion both in mice (Farbman, 1968) and mankind (Matthiessen and Andersen, 1972) and there is a good description of the typical sequence affecting differentiated muscle cells in the development of the chick heart (Manasek, 1969). Indeed it appears very widely in the animal kingdom as a mode of cell deletion in the genesis of tissue form; it is seen in insect metamorphosis (Goldsmith, 1966).

Recently we have shown that it occurs in postnatal tissues also. The gross atrophy of the adrenal cortex following withdrawal of ACTH stimulation in both physiological and experimental conditions is due to a swiftly effected surge of apoptosis as well as to atrophy of the remaining viable cells (Wyllie, Kerr, Macaskill, and Currie, 1973; Wyllie, Kerr, and Currie, 1974). Apoptosis in excess of that seen in normal glands, which presumably represents physiological cell turnover, appears within 24 hours of ACTH deprivation. A similar situation pertains in the prostate after castration (Kerr and Searle, 1973) and the uterine epithelium after withdrawal of oestrogen (Martin, Finn, and Trinder, 1973). Glucocorticoid-induced atrophy of the thymus cortex involves widespread death of thymocytes by an apparently programmed process (Munck, 1971) whose morphological manifestations (van Haelst, 1967; Abraham, Morris, and Hendy, 1969; La Pushin and De Harven, 1971) are at least in part those of apoptosis. It is evident that in these situations the number of cells in differentiated populations is controlled not only by the rate of mitosis but also by the rate of apoptosis.

Apoptosis can occur also in situations where cell populations are exposed to hypoxia or toxins, but where the environment is apparently not so inclement as to cause coagulative necrosis. It has been observed in the liver after poisoning with albitocin (Kerr, 1970) and heliotrine (Kerr, 1969) and is in part responsible for the rapid tissue atrophy caused by hypoxia (Kerr, 1971). Similarly, certain teratogens exert their effects by promoting apoptosis in specific cell groups, whilst the embryo as a whole survives (Crawford, Kerr, and Currie, 1972). The morphology of apoptosis in these circumstances is identical to that occurring in more physiological conditions.

Mechanism

Little is known of the mechanism of this mode of cell death. Studies on the embryo show that its timing may be intrinsically determined (Saunders, 1966). Cells committed to die did so on schedule even if explanted or transplanted in tissue culture many hours before the first morphological changes occur. Such committal could be reversed by exposing the cells to diffusible substances, so far not chemically defined, produced by certain rapidly growing zones. In the embryo this type of cell death occurred in cells in which DNA synthesis had stopped some time previously, in contrast to adjacent viable cell groups (Held and Saunders, 1965). A similar situation pertains in the ACTH-deprived prepubertal adrenal cortex where the population susceptible to apoptosis is restricted to the zona reticularis and reticularis-fasciculata border (Wyllie *et al*, 1974), zones in which DNA synthesis is low and where the oldest cells are most likely to be found (Wright, 1971).

Although the data concerning morphology, incidence, and mechanism of apoptosis are manifestly incomplete, we feel it is reasonable to suggest at this stage that, at least in some situations, it is a controlled, programmed event involved in tissue homeostasis. The programmed nature of the phenomenon is suggested by its stereotyped sequence, which is found regardless of the cell type affected, and—in contrast to coagulative necrosis—irrespective of the nature of the lethal stimulus. The selective loss of nuclear membrane and junctional complexes, with structural preservation of other organelles, suggests that the process is an organized one. That it may be energy dependent is supported by the striking cytoplasmic condensation, which is difficult to conceive of without invoking an active mechanism. The time course being relatively short, the appearance of only a few apoptotic bodies in a histological section could imply a significant degree of cell loss. In the adrenal cortex, where kinetic data are available (Wright, 1971), we have found the frequency of apoptosis to be of the right order to balance the rate of cell production; thus in this situation at least cell death by apoptosis is quantitatively capable of providing a means of controlling the cell population. Its incidence in so many situations where controlled cell death is a biological necessity provides further circumstantial evidence that it is a controlled process. That it can occur in situations where there is environmental perturbation, as in hypoxia and exposure to low doses of certain toxins, does not necessarily destroy the hypothesis that it is a programmed form of death. The cells undergoing apoptosis in these situations are dispersed within an otherwise viable population. It may be advantageous for a tissue placed in an inclement but sublethal

environment to possess the means for jettisoning only certain cells in the interests of conserving others in their normal orientation. What little is known of the mechanism of apoptosis in ontogenesis suggests that it is a function intrinsically programmed by the cell itself, yet not death merely through the acquisition of metabolic errors of senescence since it can in certain circumstances be revoked.

Cell Death in Tumours

There can be no question that in some tumours some dying and dead cells show the features of coagulative necrosis. Sheets of dead cells within tumour masses are a familiar histological picture to histopathologists, and there is good evidence that death is due at least in part to anoxia. Thus the width of perivascular sheaths of viable cells correlates well with the calculated range of diffusion of oxygen (Thomlinson and Gray, 1955; Tannock, 1968), and the mass of necrotic tissue can be temporarily increased by lowering the ambient oxygen tension (Schatten, 1962) or perfusing blood pressure (Schatten and Burson, 1965).

Cell loss can, however, be demonstrated even in the viable portions of such tumours (Mendelsohn, 1960; Lala, 1972a), and in tumours in which sheets of coagulative necrosis are not a feature (Lear, 1972b). In such situations cell death is described as involving single cells which show pyknotic or karyorrhectic nuclei. The ultrastructure of these has been studied only recently and in only a few types of tumour, but the typical sequence of apoptosis has emerged in human basal cell carcinoma of skin (Kerr and Searle, 1972), squamous carcinoma of the uterine cervix (Searle, Collins, Harmon, and Kerr, 1973) (fig 5), and in experimental mammary tumours (Currie, Kerr, Scott, and Inglis, 1973).

The identification within tumours of this type of cell death may be of considerable significance in our approach to the biology and treatment of neoplasia. This tentative suggestion is strengthened by the observation that tumour regression, induced by endocrine ablation, where nutrient or oxygen supply from the bloodstream can hardly be at fault, is in one experimental tumour effected at least in part by a wave of apoptosis, in the same way as withdrawal of trophic hormone stimulation induces apoptosis in non-neoplastic target cell populations (Currie, Kerr, Scott, and Inglis, 1973).

In regressing tumours there is morphological evidence for a variety of types of cellular damage, some perhaps lethal, others leading to cell atrophy, but the finding of apoptosis early in tumour regression may well concord with the view that tumour cells, whilst relatively insensitive to normal tissue

Fig 5 Squamous carcinoma of the human uterine cervix. An apoptotic body with condensed but well preserved cytoplasmic organelles and two dense nuclear fragments lies amongst intact carcinoma cells, identifiable by their tonofibrils and desmosomes. Note the absence of desmosomes in the apoptotic body. Lead citrate. $\times 10\,500$. Inset Light microscope view of the same tumour showing numerous apoptotic bodies. H & E. $\times 380$ (by courtesy of the Journal of Pathology and Dr J. F. R. Kerr).

homeostatic mechanisms are not devoid of intrinsic controls. Hopefully, further investigation of the triggers for and effectors of apoptosis might lead us to a fuller knowledge of what these controls are and how they may be manipulated in favour of the cancer patient.

Summary

Two modes of death are described, coagulative necrosis and apoptosis. They are morphologically and probably functionally distinct, and whilst coagulative necrosis has never been demonstrated conclusively as a mechanism controlling normal cell populations, apoptosis appears to be so in at least some circumstances. Both modes of cell death are found in tumours, and apoptosis is prominent in at least one hormone-responsive experimental

tumour regressing after endocrine ablation.

I am indebted to Professor A. R. Currie, Dr John F. R. Kerr, and Dr Colin C. Bird for their help in the preparation of this manuscript.

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