The Ageing, Growth and Death of Cells

by Rupert Sheldrake

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No cell is immortal. If a cell grows and divides, it becomes two cells; if it does not divide, sooner or later it dies. Multicellular organisms are not aggregates of cells in a state of exponential growth and division. Some cells divide, but most differentiate and do not undergo further division. Here I shall discuss the ageing and death of cells in vascular plants and vertebrate animals in an attempt to explore the significance of these processes in relation to growth and development, both normal and abnormal.

It is often convenient to think of cells of living organisms as maintaining a more or less steady state; but it is also easy to forget that this is an approximation, an abstraction which, if realised, would confer on cells and on organisms the doubtful blessings of eternal life and eternal youth. The realities of growth, development, ageing and death cannot be understood in terms of steady state concepts. They are directional and irreversible changes in time.

Some cells die as they differentiate, for example xylem cells in plants and keratinised epidermal cells in animals; some, such as phloem sieve tubes in plants and red blood cells in mammals, lose their nuclei; others which retain their nuclei may lose their ability to divide - as do most nerve cells. But even cells which retain their ability to divide will die if they do not do so; they senesce.

Any general hypothesis of cellular ageing must not only explain cellular ageing itself, but also the way in which some cell lines do not senesce and die out. The germ cell line is continuous from generation to generation (also many plants can be propogated vegetatively indefinitely) and some cell lines derived from plant or animal tissues can be propagated in vitro for an indefinite number of generations.

General hypotheses of ageing based on genetic mutation face two difficulties. First, in explaining the universality of the processes of ageing in non dividing cells in terms of a lethal accumulation of harmful mutations and, second, in accounting for the facts of sexual and vegetative reproduction, which show that mutations do not accumulate to a lethal extent in all cells. The alternative to a genetic-mutation hypothesis of ageing is some sort of 'cytoplasmic' hypothesis, the most recent and best known of which is Orgel's 'error catastrophe' hypothesis, which postulates that an accumulation of errors in protein synthesis leads to a positive feedback of error as the enzymes involved in protein synthesis themselves develop errors and thus produce more defective proteins1. For this hypothesis to account for the continuity of the germ line and the indefinite propagation of 'permanent cell lines' in vitro it is necessary to postulate a process of 'cellular selection' whereby error-containing cells are selected out.

Neither the genetic-mutation hypothesis nor the protein synthesis 'error catastrophe' hypothesis of ageing are supported by sufficient evidence to rule out the possibility that cellular ageing may be explicable in terms of the accumulation of cytoplasmic breakdown products, some of which might be deleterious to the cell if they accumulated sufficiently. In all actively metabolising cells, there is a turn-over of cytoplasmic constituents such as proteins and membrane lipids. More is known about their synthesis than about their breakdown in vivo; while some of them may be broken down completely, others may be broken down only partially or not at all. They must therefore accumulate.

Lipid peroxidation

One example of such an accumulation is provided by the 'age pigment' or lipofuscin granules which accumulate in an age-dependent way in the cells of many mammalian tissues4. The lipofuscin material contains lipid and protein and may be formed in autophagosomal vesicles, for example during the digestion of mitochondria; haem groups, released as the cytochromes are degraded, may catalyse the peroxidation of unsaturated lipids in the degenerating mitochondrial membranes which may cross link with each other and with denatured proteins 6. The cells seem to be unable to destroy these cross-linked polymers. Lipofuscin granules do not seem to damage cells directly except when they accumulate, as they do in certain diseases, to such an extent that they mechanically interfere with the structure and functions of the cell.

Lipid peroxidation does not always result in the formation of microscopically visible lipofuscin granules, nor is it confined to autophagosomal vesicles; it occurs in all functional cell membranes, including the surface membrane. Once the peroxidation of unsaturated lipids is initiated, by haem groups, Fe^{2+} ions and other simple catalysts in the presence of oxygen, it takes place by a free radical chain reaction. It can be inhibited by lipid-soluble antioxidants such as vitamin E and accelerated by vitamin E deficiency, ionising radiation, chloroform and ethanol poisoning and hyperbaric treatments 8,9, which can cause irreversible damage to cells.

The peroxidation of lipids within cell membranes is occurring in vivo all the time. Some peroxidised lipids may be metabolised but others, perhaps those which are cross linked to other lipids and lipoproteins may not be. The chain reaction of lipid peroxidation may be terminated by the oxidation of other substances which may themselves be damaged and accumulate. Such substances formed within the surface membrane, for example, may accumulate in situ; if they are removed from the surface membrane as the membrane is recycled by the invagination of membrane vesicles11-14 or by other means, some of them might find their way into residual bodies, but they might also be incorporated into intracellular membranes. The formation and accumulation of such substances within the outer and intracellular membranes, for example in the Golgi apparatus, endoplasmic reticulum nuclear membrane and lysosomal membranes, could well be deleterious to normal membrane functioning and could also lead to a positive feedback of damage by further lipid peroxidation, and thus to the senescence and death of the cell. The rate of ageing would be temperature dependent and would also depend on the composition, structure and functions of the cellular membranes, the extra - and intracellular environments, antioxidant levels and so on. Thus, different types of cells would age at different rates but, according to this hypothesis, all cells would be ageing to a greater or lesser extent all the time; all cells would be heading towards senescence and death.

The elimination of membranous material from cells might enable the ageing process to be retarded and there are a few examples of the shedding of membranes by cells which I will discuss further. But, in general, the only way in which cells could avoid their otherwise inevitable mortality would be by growing and dividing, thus diluting the accumulated breakdown products. Although lipid peroxidation may be the most important cause of the formation of such substances, the following general considerations could apply to any deleterious substances which accumulate with age.

Growth and division of cells

An artificially simple case is provided by cells dividing symmetrically with a fixed generation time if these accumulate deleterious breakdown products linearly with time, an amount, x, being formed per cell generation time. Successive generations contain more of the accumulated breakdown products but the increments become smaller and smaller. If the rate of accumulation is not linear, but proportional to the amount already accumulated, the content per cell will increase exponentially; and if there is a progressive lengthening of the cell generation time, there will be a greater accumulation within individual cells in succeeding

generations. With either or both of these assumptions, it can be seen that the whole population will undergo senescence and sooner or later die out.

But another type of cell division is possible, an asymmetrical division in which one of the daughter cells receives all or most of the accumulated breakdown products (becoming more 'mortal') while the other is rejuvenated, receiving little or none. The more 'mortal' of the daughter cells might die or differentiate directly, or it might divide again unequally, producing a rejuvenated cell and a cell even more 'mortal' than itself, or it might undergo one or more sequential symmetrical divisions (as discussed above) to produce a population of cells which sooner or later die (unless they can undergo further asymmetrical divisions to produce rejuvenated cells).

I shall now consider a few aspects of the growth and development of higher plants and higher animals in the light of these ideas. Dicotyledonous trees illustrate the pattern of indefinite growth that is characteristic of plants. (There are of course plants, such as herbaceous annuals, which die after they have flowered. But annuals are capable of growing for much longer than their normal life-span if they are prevented from flowering, indicating that they die because they flower and not because of an innate inability to go on growing15.) The life span of trees is limited by a variety of mechanical factors, but cuttings taken from old trees can give rise to healthy young trees, and this process can be repeated indefinitely. The growing points of the tree, the apical meristems, remain perpetually young.

Cell divisions within the apical meristems of the shoots give rise to daughter cells with different fates: some remain meristematic, others give rise to the differentiated structures of the stems and the leaves. Some of these cells die as they differentiate into vascular tissues and fibres, others, for example the leaf mesophyll and pith parenchyma, remain alive for some time, but, unless they are stimulated to divide again in a regenerative response to wounding or damage, they eventually die. The leaves senesce and fall from the tree; the pith breaks down. The root meristems give rise to the primary tissues of the root which, apart from those which divide to produce further root meristems, sooner or later die. In secondarily thickening stems the divisions of the cambial cells give rise to cells which die as they differentiate into xylem or undergo further asymmetrical divisions to produce phloem companion cells and sieve tubes. These cells eventually die and are sloughed off in the bark. Cell divisions in the cork cambium give rise to cork cells which die as they differentiate; divisions of the root cap initials give rise to root cap cells which die and are sloughed off. Thus, in the various meristems of the plant the continued growth and continued rejuvenation of the meristems is associated with the production of cells which die during or after differentiation.

Vertebrates

Vertebrates, unlike trees, do not go on growing indefinitely, nor can they be propagated vegetatively. At first, fertilised eggs undergo cleavages which rapidly increase the number of cells, but this rate of increase of cell number declines progressively as the animal develops, and as cells and tissues differentiate16. Throughout the development of the embryo many tissues and groups of cells regress and die17,18. Some of these cell deaths are associated with tissue differentiation19, some occur during morphogenetic processes20, and others may represent the regression of phylogenetically vestigial structures17, but the significance of other cell deaths is obscure17. As the animal develops, the cells of some tissues, such as nerve and muscle, differentiate and to a large extent lose the ability to undergo further division. Some of these cells die as the animal grows older and are not replaced21,22 but in the adult animal a number of other tissues continue to grow, for example the epidermis, the intestinal lining, the liver and blood cells continue to be formed. In all these examples the production of new cells is offset by cell death. Cell divisions in the basal layers of the mammalian epidermis give rise to daughter cells which remain in the basal layers and divide again, and other daughter cells

which differentiate and keratinise, dying as they do so. Cell divisions in the crypts of the intestinal villi replenish the population of crypt cells capable of further division and produce other daughter cells which move up the villi where they die and are sloughed off23. Asymmetrical divisions of the early precursors of all cells of the blood occur throughout life and give rise to further precursor cells as well as to the maturing and mature cells of the blood, all of which have a limited life span. During the formation of red blood cells24 and granulocytes25 in the bone marrow, and lymphocytes in the thymus26, considerable numbers of cells die in situ soon after they are formed. The reasons for this 'ineffective' erythropoiesis, granulopoiesis and lymphopoiesis are unknown.

The mortality of at least some of the cells which die in developing animal embryos and in mature animals may represent the price that is paid for the rejuvenation of other cells which continue to grow and divide. But unfortunately too little is known about cell lineages in animals, especially in embryos, for it to be possible to decide how general is the phenomenon of asymmetrical cell divisions giving rise to daughter cells of unequal mortality. The recognition of this pattern is complicated by the fact that by no means all cell death takes place as a result of cellular senescence. Some cells die as they differentiate and others may die because they find themselves in the wrong places at the wrong times19. Cell deaths may be controlled chemically, for example by steroid hormones: the injection of glucocorticoids can cause large numbers of lymphocytes to die27, the regression of Mullerian and Wolffian ducts is controlled by androgens and oestrogens19,28 and the regression of the lining of the female genital tract is under the control of oestrogens28. But, under the hypothesis that asymmetrical cell divisions lead to a rejuvenation of 'meristematic' daughter cells at the price of the increased mortality of their sister cells, it does not matter whether the latter die as a result of senescence, or whether they die as they differentiate or for any other reason.

Sexual reproduction

In the sexual reproduction of both higher plants and higher animals almost all the cytoplasm from which the embryo and the new organism develops is provided by the egg. In both cases, the egg cells are formed as a result of asymmetrical divisions of the egg mother cell. In the great majority of higher plants, the meiotic divisions of the egg mother cell produce four cells, three of which die. The fourth undergoes further divisions to produce the cells of the embryo sac, most of which die before or shortly after fertilisation. In some species, one of more of the three sister cells of the cell which gives rise to the egg may undergo further division to produce short-lived embryo sac cells29. In aminals the first and second meiotic divisions of the egg mother cell give rise to the first and second polar bodies, which regress and die.

It is particularly striking that in both plants and animals, only one of the progeny of the egg mother cell gives rise to an egg while the sister cells die (or if they divide give rise to short-lived progeny). By contrast, there is no comparable cell loss in male gametogenesis associated with the meiotic divisions of the pollen mother cells and spermatogonia.

The many examples in both higher plants and higher animals (and many more can be found in the lower plants and lower animals) of the production of rejuvenated meristematic, stem or egg cells by asymmetrical divisions do not of course prove that these divisions involve an asymmetrical distribution of deleterious breakdown products; but the available facts appear to be consistent with this hypothesis.

Loss of membranous material by animal cells

If the accumulation of deleterious breakdown products of membrane lipids is one of the causes of cellular senescence, the loss of membranous material might be of considerable importance in enabling cells to rid themselves of such substances. The shedding of membranous material

by living cells does not seem to be of common occurrence but can take place in mammalian cells as follows.

First, in apocrine secretions part of the cell membrane is lost. The best example, and the only one for which conclusive ultrastructural evidence exists, is in the secretion of lipid droplets by the cells of lactating mammary glands. The secreted lipid droplets are surrounded by a unit membrane derived in part from the surface membrane and in part from Golgi vesicle membranes.

Second, membrane-bounded vesicles of cytoplasm can break away from mammalian macrophages both in vitro and in vivo. This process, known as clasmotosis, is of unknown significance. Lymphocytes which are activated in immunological reactions or as a result of phytohaemagglutinin stimulation form 'tails' (uropods) which can bleb off vesiculated buds in vivo and in vitro. Again, the significance of this process is unknown. Clasmotosis is also frequently observed in cultures of fibroblasts.

Third, many types of animal viruses are budded off from host cells in membrane-bounded vesicles. The protein in the membrane of the vesicles is largely viral, at least in the case of RNA tumour viruses, but the lipids are derived from the host cell membrane35. Viral particles bounded by membrane are also budded off from the cells of a number of spontaneously cancerous tissues and from many of the cell strains and permanent cells lines which are commonly cultured in laboratories.

Tissue cultures

Many plants callus cultures can be grown indefinitely in vitro. During the early stages of the growth of some calluses, an exponential increase in cell number takes place at a rate which suggests that many of the cells may undergo a limited number of sequential symmetrical divisions before the growth rate declines but in most plant tissue cultures the rate of increase of cell number is more or less linear for most of the growth period39,40. Linear growth characteristics would be compatible with a meristematic pattern of cell division such that some daughter cells continue to grow and divide while their sister cells age and sooner or later die. Unfortunately nothing is known in detail about cell lineages within these cultures, nor are there any quantitative data on cell death. Nevertheless, dead and dying cells are by no means uncommon.

'Permanent' mammalian cell lines capable of indefinite propagation in vitro can be derived from cancerous tissues and also from cells which have undergone a spontaneous 'transformation' during culture. Diploid fibroblast cultures can be propagated, however, only for a finite number of subculturings, more (up to about 60) if the cells are derived from embryonic tissues, fewer if they are derived from mature organisms41. The number of generations through which the cells can be passed before the population senesces and dies out is reduced if the period of time between the subculturings is increased42. Fibroblasts of the mouse L strain have been observed to divide symmetrically over six to seven cell generations with a more or less constant generation time43; if the cells in the diploid fibroblast cultures also divide symmetrically, deleterious breakdown products might accumulate in the cells of succeeding generations, as discussed above, and account for the senescence of these cultures. It is impossible, however, to make any detailed interpretation of the senescence of these cultures in the absence of quantitative information about the proportions of dividing and nondividing cells, the incidence of cell death, and the extent and significance of clasmotosis within these cultures - or indeed with cultures of 'transformed' and 'permanent' cell lines.

Cancer

Malignancy must not only involve the freeing of cells from the normal controls on their proliferation, but also the avoidance of senescence by at least a part of the cell population. Many animal tumours contain a stem cell or 'meristematic' population which gives rise to daughter cells which may or may not differentiate, but which sooner or later die. There are numberous examples of cell death within cancerous tissues45-48. Some of the cell deaths can be explained in terms of an inadequate vascularisation of the tumour tissue, but in most tumours this is by no means the only cause an does not apply to all to leukaemias; many of the cells may die as a result of ageing.

Little attention has been paid to the incidence of cell death within cultures of cancerous cells and it is therefore at present impossible to know to what extent the patterns of cell division, ageing and death within these cultures resemble those within in vivo cancers. It is sometimes assumed, if only implicitly, that overall exponential growth characteristics of cell cultures mean that there is a homogeneous population of symmetrically dividing cells. This assumption is not justified: a heterogeneous population containing proliferating, nonproliferating and dying cells can also grow exponentially if the proportion of cells that die is constant with time.

It is conceivable that the loss of membranous material either spontaneously, as in certain types of mammary gland tumours, or as a result of the budding off of viruses (such as RNA tumour viruses) could play a significant role in the retardation of cellular senescence in certain types of cancer.

Effects of cell death

Very little is known about the biochemistry of dying cells. Such cells probably release all sorts of proteins, glycoproteins, peptides, amino acids, amino acid breakdown products, nucleic acids and nucleic acid breakdown products, lipids and lipid breakdown products as well as salts and other substances which were sequestered inside the cells.

It has recently been found that in higher plants the hormone auxin (indole-3-acetic acid) is formed as a consequence of cell death as tryptophan, released by proteolysis, is broken down. Dying cells in differentiating vascular tissue, regressing nutritive tissues and so on, are probably the major source of this hormone within the plant52. Other plant hormones may also be produced by damaged and dying cells: ethylene from the breakdown of methionine and cytokinins by the hydrolysis of transfer RNA52. In higher plants the normal production of hormones as a consequence of cell death and the production of 'wound hormones' by damaged cells can be seen as two aspects of the same phenomenon52.

Wound and regenerative responses in vertebrates cannot be explained simply in terms of wound hormones, but there is evidence that dying cells release substances that stimulate phagocytosis53, and affect growth and development in both normal54,55 and cancerous tissues56. And at least some of the cell deaths which occur during normal embryonic development may well result in the production or release of substances involved in the control of differentiation and development.

Dying cells may not only have a chemical effect on neighbouring cells but also a physical effect as cell to cell contacts are broken. Cell deaths within a tissue may also affect the functioning of the tissue as a whole: for example, the death of nerve cells within the brain22 seems likely to affect pathways or patterns of nervous conduction, perhaps leading to the formation of new pathways or patterns. Such cell deaths could act as a source of random change within the nervous system that might not always be deleterious57.

So little attention has been paid to the ageing and death of cells during growth and development, both normal and abnormal, that detailed information about these processes is

scarce. Where facts are few, speculation can flourish. Most of the speculations advanced in this article could be opposed by alternative speculations, but they illustrate the view that growth and development cannot be understood in isolation from ageing and death. This is by no means an original concept, but at the cellular level it provides a perspective in which many familiar facts take on a new significance and suggests a new approach to familiar problems.

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