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The changing understanding of ageing

Part 2: Body composition, metabolism and cell death

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Summary

This second of three discussions about ageing biology and diseases continues at the level of the organism, examining the relationship among body composition, late life and diseases. One view of significant age-related mass loss in humans suggests that anabolic failure is associated with various precipitating factors that may share anorexia in common. Lean mass decline with even partial anorexia should alert clinicians to monitor patients for emergence of otherwise subclinical disease. Weight or mass loss and clinical disease also may be independent, thus creating an interwoven and complex view. Recent data from the Portuguese water dog genetics model suggest that heritable factors play a role in end of life body metrics and some histological changes, and that some metric and histological changes are themselves inter-related. While widespread inflammation and hyperplasia were less frequent than expected, there exists nonetheless a disease relationship to the growth hormone (GH)-insulin-like growth factor (IGF-1)-insulin axis that requires further exploration. Oxidative metabolism and apoptosis are reviewed briefly as examples at the cellular level that may be reflected in gross ageing phenotypes, further underscoring the complex nature of many late-life diseases.

Keywords

Ageing diseases, Anabolic failure, Apoptosis, Body composition, Free radical theory, Genetics, Hormesis, IGF-1, Insulin-like growth factor, Portuguese water dog.

L'evoluzione del concetto di invecchiamento
Parte 2: Composizione dell'organismo, metabolismo e apoptosi

Riassunto

Questo secondo contributo sull'invecchiamento prende in esame l'organismo e la relazione tra la sua composizione, la vita in età avanzata e le malattie. Una teoria sulla perdita significativa di massa correlata all'età nell'uomo suggerisce che il declino anabolico è associato a vari fattori precipitanti che potrebbero avere in comune l'anoressia. Il calo della massa magra associata all'anoressia anche parziale deve allertare i clinici portandoli a monitorare i pazienti per l'eventuale insorgenza della malattia altrimenti subclinica. La perdita di peso o massa può anche essere indipendente dalla malattia clinica creando così un quadro interrelato e complesso. Dati recenti sul modello genetico dei cani d'acqua portoghesi indicano che i fattori ereditari sono coinvolti negli aspetti metrici dell'organismo a fine vita e in alcune variazioni istologiche e che alcune alterazioni metriche e istologiche sono interconnesse. Benché l'infiammazione diffusa e l'iperplasia siano risultate meno frequenti delle aspettative, esiste una relazione tra malattia e asse GH-IGF1-insulina che deve essere ulteriormente indagata. Il metabolismo ossidativo e l'apoptosi sono discussi brevemente come esempi, a livello cellulare, che possono ripetersi in fenotipi evidenti soggetti a invecchia-mento, il fenomeno evidenzia ulteriormente la

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natura complessa di molte malattie che si manifestano in età avanzata.

Parole chiave
Anabolismo, Apoptosi, Cane d'acqua portoghese, Genetica, IGF-1, Invecchiamento, Malattie, Organismo, Ormesi, Radicali liberi.

Introduction

In the first paper of this series, I examined arguments for and against some theories of ageing, and considered problems often encountered with studies of ageing. In this second paper, I look more closely at ageing from the level of the organism and below. The central ideas of these discussions continue to revolve around why post-reproductive life span occurs relatively commonly among animals, the nature of stochastic versus programmed influences on age-related diseases and the disease-related implications for clinicians. Again, I have been selectively inclusive, with primary focus on the changing views of several important ageing phenotypes.

The new scope of mass loss

Investigators recently reported an *in vivo* study of organ mass and ageing in humans (32), confirming what has been observed from studies of cadavers. Magnetic resonance imaging (MRI)-derived mass for heart, brain, kidneys, liver and spleen was obtained from 111 humans aged between 19 and 88 years. Left ventricular mass was also estimated using echocardiography. Dual-energy X-ray absorptionmetry (DEXA) was performed to evaluate body composition.

Organ masses for brain, kidneys, liver and spleen were smaller with age, but left ventricular mass was larger (32). With respect to skeletal muscle, considerable and clear literature is available and has been reviewed by Doherty (23) documenting *gradually* declining strength and muscle mass during later life in humans. However, even this well-studied aspect is not straightforward. For example, when DEXA is used to assess lean body composition, the software does not separate soft organ and skeletal muscle subcomponents of the lean mass total. By contrast, the MRI technology used by He and colleagues confirmed that both soft organ mass and skeletal muscle mass decline with age (32).

A number of interesting research questions are raised by this recent study, as follows:

- how do organ mass changes relate to clinically detectable ageing phenotypes?
- do lean mass changes relate to the decline towards frailty that is often observed in older humans?
- what expectations are implied for the ultimate histopathological outcomes of these organs?
- what is the implication of increasing left ventricular mass while other organs decline?
- do *disease-independent* longitudinal mass changes in organs-within-subject proceed at parallel or different rates?

In the case of increasing heart mass, causal hypotheses for humans might include hypertension, effects of socio-economic stressors or lifestyle choices (smoking, alcohol consumption, diet) (32). Although one could not answer all of the above-stated questions with cross-sectional data, this work encourages new hypotheses for longitudinal studies. The following sections further examine body composition and ageing in human and animal populations, beginning with more detailed consideration of skeletal muscle loss.

Sarcopaenia

Sarcopaenia is declining mass and strength of skeletal muscle during ageing (57, 60). Healthy humans experience an average 20%-40% reduced physical strength during decades 7 and 8. In one study, prevalence of sarcopaenia among 883 elderly persons ranged from 13%-24% over the ages 65 to 70, and >50% after the age of 80 (7). While muscle-related declines are greater in men, women have a longer mean life span and potentially longer periods of late-life disability, thus representing additional concern for physicians and public health providers (61). It is important to realise also that muscle mass and strength do not necessarily decline in parallel during sarcopaenia (7).

It is disconcerting that investigators have identified early skeletal muscle mass decline in some humans, with substantial change by decade five (35). Potential life span implications for individuals in this category need to be monitored by primary care and specialist physicians, and not just by geriatricians. Additional implications are that new studies need to be conducted to establish whether or how subtle mid-life changes in muscle mass and strength can be observed across animal species and what concerns accrue for their health and longevity. Study designs and length of anticipated life span will be critical to interpreting these experimental outcomes.

Sarcopaenia and healthy ageing

The next point to consider is degree of weight (mass) loss. Again, studies of humans are the most instructive, although one should note where the metric is weight and where it is one or more components of mass. An interesting review offers insights that I draw upon here (69). Increased risk of death in humans was associated with unintended loss of 5% or more of body weight over a period of 6 to 12 months (68). By contrast, given the disparities in dog breed size and breed-based longevity, equivalent estimates presently can be tenuous, especially without body composition metrics.

However, this is not the only problem. Measured lean mass declines in 'evidently healthy' humans averaged 0.3 kg/year from the *third* decade in two studies (38, 59). This early and gradual lean mass decline can be offset by spontaneous and simultaneously increasing fat mass (10, 38), suggesting that monitoring affected individuals only for total body weight could result in overlooking initial stages of chronic physiological changes and ageing disorders.

In two other longitudinal studies of healthy elderly subjects, lean mass decrements were still smaller, at 0.1-0.2 kg/year (17, 25), suggesting that the sarcopaenia process may not be completely linear. Finally, two further studies indicated that significant weight loss in the elderly is associated with increased mortality, even if the weight loss is voluntary (31, 68). This disturbing observation led to the suggestion that, given the difficulty of voluntary weight loss during advanced life, evidently effective volitional loss in elderly humans (or by prescription and supervision in elderly animals), should be monitored closely for contributing underlying disease (67).

Sarcopaenic anorexia and anabolic failure

Histologically, the bulk of skeletal muscle mass loss appears to be type II fibres (4, 23, 39, 56). Sarcopaenic loss of individual type II muscle fibres can result from a combination of influences that may be endocrine, immune, nutritional or molecular. In humans, levels of anorexia appear to be an important underlying contributor (4, 68). Veterinary clinicians also recognise the anorexia-associated phenotype, at least subjectively in non-obese older animals. It is hypothesised that withdrawal of anabolic support factors (or resistance to their action) with advancing age is a proximate aetiological event of disease-unrelated anorexia (23). Thus, the process may appear to be at least partly circular and self-perpetuating. However, if so, what causes the anabolic failure?

In humans and animals, the reasons underlying the age-related anorexia and mass loss are probably varied. Morley (46, 48) has summarised, from various studies, contributing factors that may be the following:

- physiological (oral health, gastrointestinal, sensory alterations, or endocrine-, cytokine- or pain-related)
- social (isolation)
- psychological (depression, dementia)
- economic
- disease-related.

Medical history and diagnostics directed to similar influential factors should accompany the earliest recognition of appetite decline and lean mass loss in older animals, regardless of chronological age or life functions at the time.

Exercise and mass loss

In humans, progressive resistance exercise can help manage more gradual sarcopaenia, although risk-benefit assessment for specific

exercise modalities and their alignment with age, diet and health status, need more research. Muscle strength may not parallel muscle size during resistance training, and in fact the two may change disproportionately to one another (20). This complicating disparity is not well understood at present, thus creating additional concern for health professionals who work with geriatric persons. Research of this type in non-human animals, with a goal of clinical applications, will more than likely require new technology and very creative conceptualisation.

Disease diagnostics and mass loss

Weight (mass) loss and presence of disease have been shown to be statistically independent of one another in some studies. Specifically, weight loss and death are associated, even after adjustment for baseline health (52, 69). Veterinary health screening still remains largely unaddressed through third party financial systems. As a result, veterinary clinicians may not become involved until the point of overt symptoms and presentation for disease diagnosis. Opportunities for recognition at earlier times depend heavily on whether owner attitudes are preventive rather than reactive. However, what are these 'earlier times'?

Laboratory studies of rodent populations can provide insights for research that evaluates survival of elderly humans and also can define expectations for various animal species. For example, in studies discussed in the first report of this series, male Sprague-Dawley rats *maintained relatively constant lean body mass into late life* (40, 41). In a third study, Fischer 344 (F344) rats had stable lean mass during advanced life, *until after the onset of terminal diseases in the population* (40, 41, 71). More recently, investigators evaluated cardiac output, regional blood flow and body composition of ageing F344 rats (22). In this study of conscious older rats, cardiac output redistributed to adipose and away from skin (a proportional change), principally because of increasing adipose. However, mass-specific alterations of blood supply to skeletal muscle did not change, confirming earlier observations on pre-disease lean mass stability in older F344 rats. Additionally, inverse trends in lean and fat mass within the individual human, with or without resistance exercise, have no parallel veterinary gauge except body weight and body condition scoring that best estimates fat mass. Veterinary clinicians, therefore, must remain particularly alert to body mass shifting from mid-life onward with animal patients.

Even among humans, there are complications that include longevity itself, individual ability to communicate and degree of medical supervision. In veterinary settings, issues concerning patient communication involve animal owners or caretakers and the acquisition of quantified and categorised lean mass clearly remains to be adapted for widespread use. In both humans and animals, failure to recognise early phases of mass change can result in delayed efforts to evaluate for underlying illnesses, thus compounding confusion created by known age-mass-disease inter-relationships.

Empiricism and mass loss

Should 'empirical' pharmacological strategies for managing declining body mass be discussed with animal owners and caretakers, as potential veterinary interventions? Two examples to consider might be growth hormone (GH) and insulin-like growth factor (IGF-1). Circulating levels of these two hormones certainly decline with age, thus explaining interest in using them to manage sarcopaenia (47, 49). However, costs and equivocal outcomes are problematic and side-effects can be quite serious (70, 73). In general, the potential for deleterious side-effects when using interventions that have not been evaluated critically needs to be taken seriously and considered very carefully.

Precipitous weight (mass) loss during ageing

In contrast to gradual sarcopaenia, which can lead *ultimately* to physical limitation, frailty and death, *sudden and precipitous* involuntary loss of lean mass (or weight, in some studies) is more immediately ominous and usually more directly disease-associated (4, 69). He and

colleagues studied clinically healthy individuals, which probably rules out precipitous mass losses during their study (32). An important question is whether a changing physiological status, such as serious but subclinical disease, pre-existed in any individuals in their study and whether that influenced individual outcomes. The same question arises with each individual clinical patient, human or animal. I will return to this question in the third and final report in this series.

Population applications of mass loss

Pamuk and colleagues (52) took a longitudinal experimental approach, using a body mass index (BMI) defined as weight (kg)/surface (m^2) and found that after 8 years from baseline measurement:

- men with life-maximum BMI <26 had *increased* risk for non-cardiovascular death in parallel to increasing weight loss
- men with life-maximum BMI between 26 and 29 had *increased* risk for cardiovascular death at weight losses of 5%-14%
- men with life-maximum BMI >29 had *reduced* cardiovascular death risk over 5%-14% weight loss, but not in the case of weight loss >15%
- among women, risk for cardiovascular death *increased* with life-maximum BMI <29.

In these studies, the data were controlled for age, race, gender, parity (women), smoking, pre-existing diseases that cause weight loss and pre-existing complications of obesity.

The study by Pamuk and colleagues (52) yields important considerations beyond the structured experimental results. As discussed in the first paper in this series, retrospective databases ordinarily are complex and subject to many influential or interfering variables, recognised or not. Pamuk's group has shown that great care must be exercised to study a population, prospectively or retrospectively, that is sufficiently large, well-structured and representative to support initial analytical efforts as well as subsequent sub-classifications of data. Needless to say, only well-designed and thorough studies will yield useful information. Good data require time and effort.

BMIs gauge primarily shifting of fat mass, with lean mass changes being at best crude estimates by subtraction. The validity of fat mass assessment in this context is illustrated by existing reviews (4, 52, 69), but leaves us wanting to understand more about lean mass changes involving muscle, soft organs, or both.

Implications are that changes in absolute and relative amounts of lean and fat mass, and the time courses of these changes, may be more important in animals than has been recognised.

Veterinary clinical care and experimental gerontology

While significant unintentional weight loss has a recognised association with poor clinical outcome (69), there are two related problems in veterinary medicine. Consistently objective diagnostic measures (especially for total lean mass and its two major subgroups) that could be applied on a disease-independent basis in general practice settings are not yet available. Additionally, although veterinary clinicians recognise this phenotypic pattern quite readily (at least subjectively in non-obese subjects), specific interventions in non-specific lean mass declines also are lacking.

As a result, the importance of very thorough physical evaluation, absent of access to body composition evaluation by DEXA or MRI technology, cannot be overstated. Furthermore, resistance by animal owners to earlier diagnostic screening should perhaps be explained in terms of emerging knowledge of the late-life implications of mid-life changes.

What should be understood about cell death and ageing?

Below the organism level

Rather than confusing us, the sum of body composition studies can and should lead us to understand ageing as an integrated but variable process. It is appropriate to consider recognising ageing-related health problems at earlier, less macroscopic stages that may not be recognised in non-invasive studies. Using post-mortem study designs, additional insights can be acquired.

Body metrics at death

In an ongoing study of Portuguese water dogs (The Georgie Project, University of Utah), genetics researchers are conducting post-mortem examinations. In a recent report from this study, 21 of 51 whole body or organ metrics (weight, length) correlated significantly and inversely to age at death, while two correlated positively. Among these 21 metrics, 9 were musculoskeletal measures and 7 were tissues derived from the embryonic anterior foregut (14).

Given what is known about lean body mass and late-life survival trajectories, an inverse relationship between age at death and muscle mass is not surprising (40, 41, 71). However, while embryonic foregut-derived tissues clearly are soft tissue contributors to lean mass, elucidating developmental relationships to age at death will require further research. The latter idea is intriguing, given the growing number of reports across species that link later-life health with aspects of early development. Well-known examples include studies of humans that demonstrate the following (5, 6, 12, 26, 50, 58):

- association between low birth weight (men and women) or low rate of weight gain during infancy (men) and future coronary heart disease
- association between low birth weight and future insulin resistance, especially in the case of replete or excess post-natal nutritional status.

While databases that would support similar inferences in animals are mostly lacking, the efforts in this direction for humans are encouraging (21, 30). For the future and further development of veterinary health-predictive databases, it will be helpful to overview the cell and its activities, especially cell death, to better understand relationships among morphological observations and life outcomes.

Body morphologies at death

Like death of organisms, cell death can occur from extrinsic or intrinsic causes and can be random or programmed in origin (16). Just as diverse cellular events mark ageing and death, so do diverse mechanisms for repair of insults exist. Important components of insult-response capabilities include the following:

- the nature of repair mechanisms
- the balance between insult and repair
- the relationship of insult and repair to senescence and diseases of ageing.

The balance among these attributes relates directly to cellular outcomes. Can effects of these attributes be considered on a more practical level?

The practical problem is that clinical, biochemical, or histological outcomes do not always occur in the kind of biological vacuum that readily permits unequivocal interpretation. Fundamentally, this is because living organisms have complex and interwoven metabolic systems that are dynamic by nature. Thus, in a given post-mortem evaluation, pathologists and clinicians can be confronted with gross and histological outcomes that do not align with clinical and biochemical evaluations. At least at the histological level, it will be beneficial to better understand morphologies that may initially seem unrelated to problems that are recognised in the clinic, laboratory, and post-mortem room, or to apparent cause of death.

Genetic evaluation of ageing and death

In a recent report of post-mortem findings in 145 Portuguese water dogs, histological evaluation of 27 tissues, consistent across all dogs, revealed pathological changes in numbers ranging between 2 and 12 within dog (median 7-8) (14). The traits observed were examined for heritability and for correlation to age at death.

Significantly heritable histopathological traits included liver (fibrosis), pancreas (fibrosis), digestive tract (lymphocytosis), bone (trabecular osteoporosis) and thyroid (atrophy).

Indications of heritability were also seen when all tissues or organs were combined into one category, to increase statistical power. In the latter evaluation, haemosiderosis, atherosclerosis and age at death were notable. As the study database becomes larger, these preliminary observations will be re-examined for robustness. Those that continue to segregate in the

study population should eventually become targets for identification of involved genes.

The individual histological traits that occurred frequently and *correlated most significantly and positively to age at death* were found in kidney (glomerulosclerosis) and spleen (haemosiderosis). A frequent and significant *negative correlation to age at death* was found for lung (congestion). Clustered relationships to age at death were also found, with the emergence of two trait groupings. The first grouping included leukocytic infiltration, fibrosis and atrophy, all unsurprising reflections of the inflammatory process. The second grouping included the proliferative traits adenoma and hyperplasia, along with haemosiderosis. Adenomas and hyperplasias are benign proliferative changes, but their relationship to haemosiderosis is interesting. Effects of microhaemorrhaging are a possible explanation, but further evaluation is needed.

The data also yielded early evidence of *relationships among histological and morphometric data*. The most potentially enlightening of these was a negative correlation between small intestine length and age at death. The possible involvement of the IGF-1 gene in this association is unmistakable, given that the canine IGF-1 gene explains about 50% of breed size variation (64) and may influence organ size. For example, when large and small dog breeds were sorted by IGF-1 haplotype (small or large), breed incidence of some diseases also segregated (13). IGF-1 pathways are conserved over very long evolutionary distances. Numerous biological functions are reflected (in this instance, for example, stress response). These observations suggest research avenues into the role that IGF-1 may play in disease frequency and longevity. Particularly interesting is the size-longevity relationship that involves expression of IGF-1 haplotypes (13) and the physiological nature of stress effects. This important regulatory and stress-response pathway will serve as a guiding example through the third paper of this series.

Metabolic stressors and cell death

Given the range of complexity among possible insults to cells, possible cellular responses and proximate causes of cell death, biochemists and molecular scientists are curious about what happens when subcellular organelles and their metabolic activities are perturbed by intrinsic or extrinsic events. Clinicians, on the other hand, are primarily interested in whether or how these processes affect patient health and longevity and where beneficial intervention is possible. The problem for busy clinicians is reviewing and understanding the enormous volume of often-conflicting results of molecular studies. Remaining mindful of some overarching ideas related to stress response will be most helpful.

Implications of hormesis

Beginning with the concept of hormesis, which was discussed in the first paper of this series, one might consider a few broad principles that influence cell death. Hormesis states that 'modest environmental stresses frequently enhance the average life span in a population' (direct quote from reference) (43), thus coupling stress response and longevity. A few examples of corollary questions with research applications might be as follows:

- What stress, when, how applied?
- What 'dose' of stressor? What measures of 'effect'? What assay methods?
- What species, breed/strain, age?
- What population genetics are involved (wild-type, laboratory strain, knock-out, knock-in)?
- What experimental setting, population size and structure, and environment?
- What statistical methods and by what justifications?
- Which laboratory? What vested interests, if any?
- What effects at the metabolic level?

When research and clinical environments are viewed in this way, it is not difficult to understand the degrees of confusion that can frequently be observed among results of similar studies.

From the above, it is evident that ageing research and clinical landscapes must extend beyond disease cataloguing if ageing biology is to be better understood in the clinical arena. Can one make some brief but useful clarifications? Kapahi and colleagues, from the laboratory of the eminent biologist Thomas Kirkwood, have indicated that 'the gene network regulating the cellular response to stress is functionally important in ageing and longevity' (direct quote from reference) (36). One should not underestimate the critical importance of the idea that genetic capacity for stress response underlies much of ageing biology and medical intervention. Genetic capacity in this sense is not just a simple matter of gene-and-product in linear cause-and-effect relationships. Rather, the biochemistry of cell ageing, as with organ and organism ageing, is governed by a complex and interwoven network of stress- or insult-response genes (36).

Kapahi and colleagues also bring to light a very important direct consequence of the network precept, namely: various cellular stressors cause damage by distinct actions, with multiple possible responses available to the cell. Thus, cell-specific life spans are not usually determined by singular intrinsic events (36). Furthermore, the fact that some of these fundamental stress responses are highly conserved (in degrees of species-unique fashion) speaks convincingly about their importance in the cycle of life itself.

Considering whether the design and outcome of a metabolic experiment sufficiently support the conclusions that are offered allows a reader to be informed (and, sometimes, forewarned). As examples, one might consider just two (of many) molecular processes that are fundamentally important in cellular, organ and organism ageing. Rather than attempting to conduct exhaustive (and impossible) reviews, focus instead will be on work that heralds possible changes in how these sub-disciplines are viewed.

New thoughts on oxidation

Described briefly and clearly by Simon and colleagues (63) and paraphrased here from their writing: the somewhat collective term 'reactive oxygen species' (ROS) refers to small, very active molecules such as:

- hydroxyl (OH^-), alkoxyl (RO^-), or peroxyl (ROO^-) radicals (*short half-life*)
- superoxide (O_2^-), or nitroxyl (NO^-) radicals (*medium half-life*)
- some non-radicals, such as hydrogen peroxide (H_2O_2), hydroperoxides (ROOH) and hypochlorous acid (HOCl).

These metabolic products can damage DNA, protein and lipids and, of course, lead to cell death. Mitochondria are the principal (but not exclusive) intracellular source of ROS, where they are generated by normal activity in cellular aerobic respiration pathways (72). ROS in cells are counteracted normally by endogenous antioxidant molecules, such as reduced glutathione, catalase and superoxide dismutase (63), acting in general to convert ROS to non-damaging metabolites, but often not at 100% conversion. Hence, a certain amount of ongoing damage is implied.

Oxidative damage and its consequences have been accepted for many years by many scientists as a (or *the*) fundamental underlying driving mechanism for ageing and longevity. However, emerging evidence ultimately may characterise oxygen radical metabolism somewhat differently. For example, Simon and colleagues point out that ROS also may act as necessary messengers in cellular signalling and transcription activation (63), as several studies to date have shown (18, 33, 37, 62).

In a recent and potentially very enlightening study, integrated imaging methods were used to evaluate temporal signals following oxidative stress (15). Skin carcinoma cells were treated in culture with zinc (as sulphate). Three chemiluminescent agents were added to detect peroxide, mitochondrial membrane potential, or mitochondria-targeted redox potential (the latter two being direct indicators of stress and damage). The data indicated that peroxide sensing occurred after, not before, sensing of mitochondrial damage, suggesting that detectable oxidative damage might be secondary. While the authors point out that additional studies are needed to confirm that the results are not consequent to luminescent-

related time-delayed expression, the results could change significantly the view of oxidation theory and ageing (i.e. initial damage may cause ROS leak rather than result from it).

Bearing in mind that the present discussion does not review more than this tiny fraction of the extensive literature surrounding oxidative biology, one can nonetheless begin to appreciate that variable and often biologically or environmentally circumstantial outcomes of these studies might be the rule rather than the exception.

Roles of apoptosis

Apoptosis is programmed cell death, to which all cells of multi-cellular animals are subject in given circumstances (2). Cell elimination occurs for a wide variety of reasons, as diverse as genetic regulation, maintaining cell phenotypes and physiological outcomes, embryonic development and many disease states (2). As with oxidation, this discussion cannot hope to be all-encompassing but it is instructive to look at some of the primary factors and influences in order to understand the role of apoptosis in the ageing process.

Apoptosis has a recognisable histological appearance. It is a controlled intracellular event whereby the nuclear chromatin can be seen to condense and then fragment. The cytoplasm condenses and the cell membrane remains intact while the cell becomes smaller. The nucleus fragments and small membrane-bound apoptotic bodies are released interstitially and are generally phagocytosed rapidly (42). There is little or no accompanying inflammatory response, mainly because the process is rapid from start to finish. This rapid and non-inflammatory nature of apoptosis often results in histological 'invisibility'. That is, the process starts and concludes quickly, with little post-event evidence.

The organisation of apoptosis involves a number of interacting pathway components that are conserved from the nematode *Caenorhabditis elegans* to humans, arguing strongly that this is an ancient programme for cell removal. In overview by Lodish *et al.* (42), proteins in the vertebrate apoptosis pathway, having homologues as far away as primitive invertebrates), can be classified as follows:

- regulators that promote or suppress apoptosis, being at times advantageous and at times deleterious
- adaptors that tend to promote activation of the downstream pathway and that may be suppressed by a range of trophic factors
- effectors (executioners) that activate and lead to cell degeneration; many but not all of these are a family of cysteine-aspartate proteases called caspases.

Apoptosis is 'fine tuned' in many ways. There are pro-apoptotic factors that influence the process in one direction and anti-apoptotic factors that influence in the other. Some cellular factors are 'Janus-faced' in that they may exert promoting or suppressing influence under different circumstances. An example of seemingly mutually exclusive functions, ROS play a role in apoptosis induction (62) in many types of cells (reviewed by Simon and colleagues) (63). This induction process seems to be associated with simultaneous release of cytochrome *c* through opened pores (possibly from oxidation) in mitochondrial membranes (29). Mitochondria are damaged in the process, thus being both source and target of ROS (63). Another recognised example is an anti-oncogenesis protein called p53 (65) that serves a regulatory function both for the proliferation and death of cells by acting with other death regulators at points of transcription.

The programming importance of apoptosis in the embryo

Primordial germ cells must form and migrate from the allantois to the genital ridge or germinal ridge, developing ultimately into the gonad. Multiple endocrine signals, along with paracrine and chemotaxic factors, guide this migration and 'straying' cells fall subject to apoptosis (27, 28, 44, 51, 65). This controlled process continues through and beyond subsequent gonadal development (19, 53). Furthermore, apoptosis is an integral part of subsequent mitoses and meioses as embryonic and foetal development continue (74).

Broad conservation of apoptotic mechanisms in development indicates a powerful role in

shaping the individual. As apoptosis and proliferation proceed simultaneously in the developing embryonic gonad, the extent of the resulting changes are such that male and female germ cell losses have been estimated at 50%-67% (male) (1, 34, 45, 55) and >67% (female) (3, 8, 9, 11, 24, 54), with only 5%-6% of the original ovarian germ cell pool remaining by female puberty (66).

All of these observations illustrate the massive impact that normal apoptosis of development has on future individual germ cell characteristics and thus on the evolution of populations and species (65). The fact that various metabolic processes influenced by apoptosis have apparently conflicting activities, being sometimes 'positive' and sometimes 'negative', is not abnormal. It simply reflects the complexity of development and of ageing.

Concluding comment

The first two papers of this series have explored, with examples, some changing thought with respect to major aspects of ageing. These ideas range from body mass changes and lean mass decline that eventually signals oncoming frailty and the death trajectory, to mechanisms, such as oxidation and apoptosis, that will influence how the individual changes from conception onwards. These processes in the individual, echoed across the group, also signal how populations might become stable or unstable and, therefore, how species evolve. Including data from an ongoing study of genetics and ageing-related diseases gives the reader a sense that linking the cell, individual and population will be possible as new studies are completed. I approach the third and final paper in this series considering new perspectives on the reasons for ageing and diseases of ageing.

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