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BIOMEDICAL CHALLENGES OF HUMAN SENESCENCE: A REVIEW
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ABSTRACT

Objective: **To summarise and discuss the progress made in the study of human senescence over the past one hundred years and assess the achievements to date.**
Data sources: **Published original research and reviews during the past one hundred years.**
Study selection: **The summary focused on those contributions that tested the various hypotheses that attempt to identify and explain the factors that are involved in the ageing process.**
Data extraction and synthesis: **Online and manual library searches provided a body of data on which the summaries and discussions were based. Specific questions were addressed: Why does ageing occur? What are the key mechanisms? To what extent are genetic and environmental factors involved in the ageing process? How does the immune system behave during ageing and especially against infectious agents? Answers to these questions were discussed against the background of major improvements in life expectancy in most parts of the world except for sub-Saharan Africa where the HIV/AIDS pandemic has reversed the trend.**
Conclusion: **Biological and clinical studies over the past century clearly reflect a better understanding of the major factors involved in human senescence. It is appreciated that human life expectancy has improved dramatically over the period through achievements in public health, therapy, nutrition and general living standards. A great deal remains to be done through multidisciplinary research before the quality of life can be improved further.**

INTRODUCTION

Understanding the biology of human senescence is a major scientific challenge for both biology and medicine. The questions at issue are not new. Two critical factors have changed the context within which the relevant questions are posed. First, an enormous demographic revolution is in progress with far-reaching consequences for all aspects of human life(1). Life expectation has doubled since the mid 19th century from around 40 years to nearly 80 years in developed countries(2). This has been a global trend with some of the most rapid change currently occurring in the developing countries. The change results from declines in the birth rate and in early mortality rather than from any intrinsic increase in human longevity which remains largely unaltered. The upshot is a redistribution of the population among the different age categories with the population structures dominated by the old in developed countries and by the young in the developing countries(3).

A second major change has been an advance in the scientific tractability of ageing research which until recently has been widely criticised by some as just too complex for serious scientific investigations(4). Recent theories provide a sharper focus questions and new techniques provide the experimental means to investigate complex, multicausal phenomena in considerable detail.

This article summarises progress that has been made over the past one century and assesses the scale of the task that remains. Some of the major questions include: Why does ageing occur? What are the key mechanisms? What roles do genes play? How many genes are involved? What is the role of the non-genetic factors such as nutrition and exercise? Are gene-environment interactions important? Is there such a thing as normal ageing? Are there shared mechanisms between different age-related diseases? Can the diseases of old age be delayed or prevented? How does the immune system interact with HIV at different stages of ageing?

DEFINITIONS AND DEMOGRAPHY

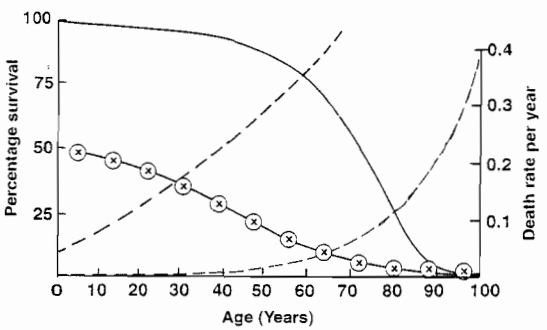
The terms 'senescence' and 'ageing' will be used interchangeably and both refer to the general observation that for the individual human, the passage of chronological time is eventually associated with a generalized impairment of physiological functions, a decreased ability to respond to a wide range of stresses, an increased risk of age-associated disease as well as an increased likelihood of death.

The term 'lifespan' will be used to refer to the duration of life of an individual. Human lifespan is treated as a quantitative trail like height. "Average Lifespan" and "maximum lifespan" are statistics relating to the distribution of lifespan within a population, "Longevity" will be used as a more general measure to describe the distribution of lifespan within a population when early deaths due to non-age-related factors are excluded. This use is not precise but is intended to cover the fact that there is an intrinsic distribution of biological lifespan potentials within a population.

The pattern of mortality (Figure 1) reflects that the major causes of deaths in developed and developing countries today are cardiovascular diseases and cancer, respiratory and infectious diseases such as malaria, pneumonia, TB and HIV/AIDS respectively. In spite of major changes in the principal causes of death since 1825(5) the exponential pattern remains largely the same.

Figure 1

Human survivorship curves and death rates



_____ Survivorship curve in developed countries
-------- Death rates/years in developed countries
X____X____ Survivorship curve in developing countries
---------- Death rates/years in developing countries

Why do we age? An area of significant progress has been in our understanding of the evolutionary basis of senescence, as it occurs not only in humans but as a general feature of higher organisms (6-8). The key role of the evolutionary theory of senescence is to identify the types of genes that might be responsible for ageing, how many they are likely to be and how they might have been modulated by natural selection to bring about difference in ageing processes especially in the rate of ageing in different species. The evolutionary puzzle is to explain why senescence occurs inspite of its obvious disadvantages. The age-related increase in mortality which is usually accompanied by a decline in fertility is clearly detrimental to Darwinian fitness. Furthermore, not all species show intrinsic rises in age-specific mortality(9) requiring that we explain why senescence is present in some species but not in others.

The first contribution of evolutionary theory is to dispel the notion that senescence is under active genetic control(10). Arguments based on the idea of advantage to the population as a whole for example to prevent overcrowding are unsatisfactory in a number of ways: First, because most animals in their natural environment die from accidental causes before major senescent changes become apparent(11). Second, because the argument is group-selectionist and requires that we peg a tenuous advantage to the groups against a clear disadvantage to the individual. Thus, it is quite clear that there is a recurrent tendency to seek to interpret ageing as a programmed process regulated by intrinsic "developmental clock" when none have been identified(12).

Evolutionary theory tells us instead that the widespread occurrence of ageing is not plausibly explained by the observations that:(i) natural selection is relatively little concerned with events that happen later in the lifespan and (ii) the acquisition of greater longevity usually involves some cost. From these general principles, three broad categories of genes can be causally involved in senescence. These are:

(i) Maintenance and repair genes
(ii) Pleiotropic genes
(iii) Late acting genes

The first category comprises genes that regulate intrinsic processes of somatic maintenance and repair. Maintenance processes range from intracellular systems such as DNA repair and antioxidant enzymes to systems requiring continuous cell turnover such as the immune systems and the epidermal layers of the skin. High levels of maintenance confer extra protection against intrinsic and extrinsic stressors and may delay or prevent the accumulation of certain kinds of somatic damage that are thought to cause senescence. Extra metabolic costs will be incurred and these may directly or indirectly reduce the resources available for other essential functions of the organism including investment in reproductive effort. The "disposable soma" theory of senescence (13-15) recognises the essential nature of this trade-off and predicts that the optimum strategy is to invest only as many resources in somatic maintenance as are necessary to remain in reasonably sound condition through the natural expectation of life in the world.

A second category derives from the more general idea of negative or antagonistic 'pleiotropy'. It has been recognised(16) that genes which confer advantages in the early stages of life, but prove deleterious later, will nevertheless be favoured through selection if the late harmful effects are outweighed by the early benefits. The differential weighting that results from the declining force of selections with age will ensure that this is often

the case. Thus, there may be a range of trade-offs mediated through pleiotropic gene action in addition to those involving the genes for somatic maintenance. The complementarity between the first and the second categories is considerable: e.g. selection for rapid embryonic development may be at the expense both of resources required for maintenance and repair and also of other factors affecting durability of the adult soma.

Thirdly, there may be mutations that have purely deleterious effects but have their actions at such late ages that the power of natural selection to remove them is too weak. Such mutations may be effectively neutral in the wild environment although strictly all that is required is that the rate at which existing mutations are removed by any weak selection force that may act against them. 'The "mutation accumulation" theory was proposed originally by Medawar(11). Martin *et al.*(8) have recently introduced the terminology, "public" and "private" to distinguish genes associated with ageing that are likely to be either shared or individual respectively. Genes involved in regulating cell maintenance are likely, to be the most public since intracellular maintenance processes tend to be similar even in otherwise distinct genotypes. At the other extreme late-acting deleterious mutations may be highly individualistic or private since the fate of these alleles will be determined largely by random genetic drift. Non- maintenance pleiotropic genes are likely to be intermediate, public within a population or species but not necessarily, shared between species.

IMPLICATIONS OF THE EVOLUTIONARY THEORY

A number of predictions follow from the evolutionary theory. One, it is predicted that multiple kinds of genes may contribute to senescence and that the number of such genes may be quite large. Two, the divergence of species longevities can be readily understood in terms of differences in the prevailing levels of environmental mortality. If a species inhabits a dangerous ecological niche, mortality levels will be generally higher and the force of natural selections will decline faster with age. For each of the gene categories considered in the previous section, this is expected to result in more rapid senescence. On the other hand, any adaptation that confers a reduced level of risk prepares the way for evolution of increased longevity. For the "mutation accumulation theory" a reduction in risk exposes the deleterious alleles to new selection either to eliminate them or to further postpone their time of action. In the "disposable soma" and "pleiotropic gene theories" a reduction in risk may alter the balance in favour, for example, of greater investment in maintenance. Humans have the greatest longevity of any mammal. It is easy to see how progressive adaptations leading to greater brain size and increasingly social living have reduced the level of environmental risk and provided the evolutionary basis for increasing longevity.

The most explicit predictions follow from the disposable soma theory(17). In this context, the polygenic nature of senescence is specifically linked to the multiple maintenance and repair systems that protect against accumulation of somatic damage, since the optimality principle that underlies the theory applies equally to each of them. This leads us to expect that on the average, the longevity assured by individual maintenance systems will be similar (Figure 2). This is because if the setting of any one mechanism is unusually low so that failure occurs chiefly from this cause alone, then selection will tend to increase this setting. Conversely, any mechanism that is set too high may incur disproportionate metabolic costs and selection will tend to reduce it.

Figure 2

Polygenic control of lifespan through the action of somatic maintenance functions as predicted by the disposable soma theory. On the average, the longevity, assured by each specific maintenance functions is predicted to be similar but variance within the population means that individuals may vary in the actual levels of the different functions

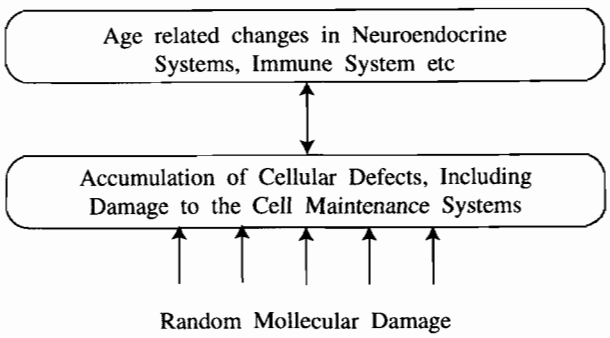
Somatic maintenance function	Longevity assured
DNA repair	O----------
Antioxidants	O----------
Stress proteins	O----------
Accurate DNA replication	O----------
Accurate DNA synthesis	O----------
Accurate gene regulation	O----------
Tumour suppression	O----------
Immune function	O----------

Despite this general harmonisation some variation is to be expected. The optimisation process is not exact and as the optimum is approached, the selection differential decreases. Furthermore, there may be gene-environment and other interactions that tend to preserve some polymorphism within the population. Individuals with markedly reduced longevity assurance for a particular maintenance function may display characteristic pathology leading to early death as in the certain inherited DNA repair deficiencies or progeroid conditions such as Werner's syndrome.

A further prediction of the disposable soma theory is that the process of senescence is itself stochastic (Figure 3). The overall rates of accumulation of damage are regulated through genetic settings of the various maintenance functions but the individual events on which these accumulations are based are random.

Figure 3

Stochastic mechanisms are predicted to be the primary cause of senescence producing a gradual accumulation of random damage to cells. This eventually leads to a range of effects on cell and system functions



Stochastic effects will be most apparent where the numbers of initiating events for an age-related change are small as in the formation of a tumour but in all cases will contribute to the intrinsic growth in variability that is one of the hallmarks of senescence. Lastly, it is implied that senescence may be to some extent malleable. The idea that senescence results from stochastic accumulation of somatic damage indicates that in principle aspects of the ageing process may be altered by, modifying the exposure to damaging agents and/or enhancing maintenance.

Genetics of human life span: The genomic analysis of human longevity seeks to identity specific genetic factors involved in senescence(18). It has long been known that there is a heritable component to human lifespan. The analysis of human heritability of lifespan was initiated by Pearl and Pearl(19) and more recently, a study of lifespan records of Danish twins(20) reported heritability estimates ranging between 20% and 35%.

Several kinds of genetic factors may be involved. The three categories of genes discussed earlier are of greatest interest. In addition there may be genes that confer susceptibility or resistance to specific age-related diseases. In order to avoid confounding true longevity genes with those that shorten life through pathology that is only indirectly associated with senescence, the major interest is in the genetic basis of above-average or extreme lifespans. Two broad strategies are available(18). One is the candidate gene approach using association or case control methodology. The other is the sib-pair or kin analysis method designed to detect loci that segregate with the trait of interest. The latter approach has the advantage that no hypothesis is required about the genetic determinants of the trait under investigation, but it requires the identification of a sufficient number of sib-pair or kin groups sharing the trait in question. Such groups are rare for the trait of extreme lifespan e.g. survival to age 100 and this approach awaits application.

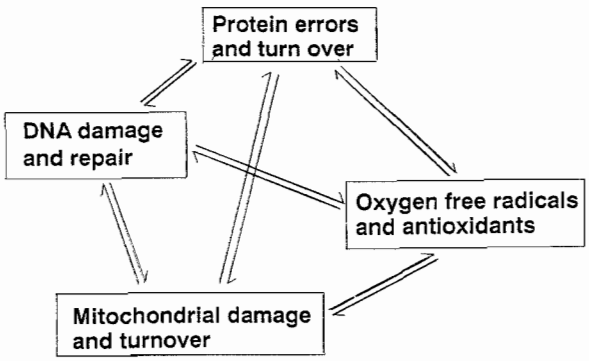
Case control studies comparing human centenarians ("cases") with younger individuals from the same genetic background ("controls") have been applied to HLA(21) and apolipoprotein E(22,23). These loci were each selected for their known association with age-associated diseases. The largest of these studies(23) compared a total of 338 French centenarians with controls aged 20-70 years. For apolipoprotein it was found that the type 4 allele that is associated with increased risk of both cardiovascular disease(24) and Alzheimer's disease(25) was less frequent in centenarians as might be expected. For the angiotension converting enzyme, it was found that the D variant which predisposes to coronary heart disease(26) was surprisingly more frequent among centenarians than among the controls, suggesting possibly that the D variant confers some pleiotropic benefits on late-life survival.

Mechanisms of senescence: Information on the molecular, cellular and systemic changes that occur during human senescence is abundant. However, a great deal still needs to be done to establish how the various mechanisms operate individually and collectively. One of the major difficulties is to disentangle primary from secondary factors. Of central importance is the need to apply intergrative approaches that combine detailed knowledge of the normal functions of the body with analysis of the diverse ways that stochastic mechanisms can contribute to the disruption and eventual breakdown of these functions. Random alterations in molecules and cells are particularly difficult to detect and it is only recently that techniques such as the polymerase chain reaction (PCR) have facilitated some progress.

Even within the cell, there is an important need to integrate our understanding of the different candidate mechanisms that have been suggested for senescence. Four of the principal mechanisms that have been proposed are accumulation of somatic mutations, accumulation of oxidative damage, accumulation of aberrant proteins as well as accumulation of defective mitochondria. While each of these mechanisms has been advanced as a hypothesis on its own, it is clear from the disposable soma theory that all the four operate in parallel(17). If this is the case, then there may be important synergistic interactions (Figure 4). This idea has been recently tested in a series of theoretical models (27-29) the latest of which(29) develops a network theory that incorporates the following features. Accumulation of defective mitochondria, effects of aberrant proteins in protein synthesis, the damaging actions of oxygen free radicals, the protective role of antioxidant enzymes like superoxide dismutase and the turnover of proteins by proteolytic scavengers.

Figure 4

Network of interactions among the various primary hypotheses concerning the intracellular mechanisms of senescence



This model has been shown to predict many of the observations and experimental findings reported from ageing cells and organisms including:

a) A sharp rise with age in the fraction of inactive proteins, thus explaining the frequently observed loss of specific enzyme activity.
b) The development with age of only a slight rise in the fraction of erroneous proteins, thus explaining why a decline in enzyme specificity is only rarely observed.
c) A significant increase with age in protein half-life.
d) A decrease with age in the mitochondrial population.
e) An increase with age in the fraction of defective mitochondria.
f) An increase with age in the average rate of free radical production per mitochondrion.
g) A fall with age in the average level of ATP generation per mitochondrion.

Understanding the role of cellular ageing *in vivo* is another major challenge for integrative study. The *in vitro* model of cell replicative senescence pioneered by Sith and Pereira has elucidated the mechanisms limiting cell proliferation(30). Until recently the link between *in vitro* and *in vivo* senescence rested mainly on the observations of (a) an inverse correlation between the number of *in vitro* cell doublings and the age of the human cell donor (31) (b) a positive correlation between species longevity and cell doubling potential (32). More recent work, using a novel marker for cell senescence has shown that senescent fibroblasts and keratinocytes can be detected during the ageing of skin *in vivo* and such studies need to be extended in order to give a better understanding of the relationship between cellular changes and senescence of organs (32).

Two further models illustrate the potential and challenge for integrative analysis of cell ageing. One is the phenomenon of telomere shortening as originally observed in human cells *in vitro* (33). The other is the human genetic condition - Werner's syndrome which is associated with an accelerated senescence phenotype. Telomere shortening results from inactivation of telomerase in somatic cells and leads to the gradual loss of telomeric DNA sequence. Telomerase is active in germ cells, immortalised cell lines and cells grown from many malignant tumours (33). Thus telomerase displays exactly the attributes predicted from the disposable soma theory. Growing evidence suggests that telomere shortening may play a role in tumour suppression but it is not yet clear to what extent it contributes causally to human senescence. Telomeres do not appear to shorten in post-mitotic tissue but these cells also undergo senescent changes. Thus, the telomere hypothesis requires integration with other mechanisms that have been implicated in cell senescence, particularly senescence of post-mitotic cells such as neurones.

Werner's syndrome is a rare recessive disorder that affects about ten in a million people and produces a complex phenotype characterised by premature development of a variety of age-related diseases such as arteriosclerosis, type II diabetes, cataracts, osteoporosis and tumours. Many of the features of Werner's syndrome are consistent with a defect in cell proliferation and cells grown from Werner's syndrome patients show reduced replicative potential and increased chromosomal instability when compared with cells from age-matched controls. Recently, the gene responsible for Werner's syndrome was identified by positional cloning and it appears to clone for a DNA helicase(34). This discovery suggests that the mutant gene may result in an accelerated accumulation of DNA damage in dividing cells and also suggests why post-mitotic tissue is relatively spared in Werner's syndrome patients. This discovery offers an important route to investigating the interrelationship between cell proliferation and senescence pathology even though there is no particular reason to suppose that in normal individuals helicases are primary determinants of the rate of senescence.

Ageing and disease: Many human diseases show progressively increasing age-incidence curves. The precise nature of this relationship has been difficult to define (35,36). Hardly anyone dying at the age of say 90 or above is without pathological changes in the heart, brain, nervous system etc. even though they have not been diagnosed as having a specific disease. This partly reflects the arbitrary cut-offs required for clinical diagnosis. It also reflects what appears to be an intrinsic feature of the ageing process i.e. senescence is normal and yet by its very nature, it involves the production of abnormality. During senescence, there is a gradual corruption of the somatic genotype together with an

increasing accumulation of defects that result in a stochastic broadening of phenotype. Some age-related diseases show greater incidence at older ages presumably because the causative mechanisms require time such as in the accumulation of somatic alterations leading to malignancy in a cancer cell. Even in cancer, there may be a fundamental link to the ageing process. In rats, cancer incidence rates rise much faster than in humans so that in spite of a 30-fold difference in longevity between the two species, the lifetime risks of developing a malignant tumour are similar(37).

Lifestyle: Nutrition and exercise are examples of lifestyle factors that may have some effect on human senescence. Both can work in either direction. Under-nutrition in humans particularly ante-natally results in increased epidemiological risk of a number of late-life diseases including hypertension and cardiovascular disease(38). Conversely, calorie restriction post-weaning produces a significant increase in the lifespan of laboratory rodents that appears to involve a general upregulation of a number of maintenance systems (39,40). Exercise can increase mechanical wear and tear contributing to the early development of age-associated conditions such as asteoarthritis and in theory leading to the generation of a greater flux of free radicals derived from mitochondrial metabolism thus potentially contributing to mitochondrial damage. Recent work has shown that regular exercise can also ameliorate age-related changes in the mitochondria of ageing human muscle(41). Mitochondrial defects accumulate with age, particularly in post-mitotic tissue. Theoretical modelling of this process indicates that a key determinant of the rate of accumulation of defective mitochondria is the mitochondria turnover rate(42). Exercise may increase turnover rate, thus providing a mechanistic explanation for delayed mitochondrial ageing in muscle.

Future prospects: Human senescence is increasingly being recognised as a research priority both for its intrinsic interest and for its importance to society. The relationship and influence of environmental factors on innate mechanisms are clearly complex especially in a tropical environment full of infectious pathogens. The challenges are immense. It is clear that there is a great need for a multidisciplinary effort in order to capture all the underpinning discoveries arising from molecular and cellular gerontology. Utilisation of animal model systems such *Drosophila melanogaster*(31) will reduce the cost and increase speed of discovery since several mechanisms operating in such animal models are likely to be shared with man.

In summary, considerable speculation attends discussion of the future of human longevity. Demographers can predict accurately based on current birth and death rates the shape of the population curve. It is clear that the major gains in life expectancy have been made already through reductions in early mortality. The changing patterns of human survivorship and reproduction have created a novel situation for our species in which our present day life history is far from evolutionary equilibrium. Consideration of other issues such as the HIV/AIDS pandemic and its impacts on the population pyramid further distabilise the ageing process. The realistic and credible goal of research on human senescence is to create a deeper understanding of the mechanisms responsible for infirmity, disability and diseases in old age and to harness this knowledge to enhance and extend the quality of human life. Understanding the biological basis of the ageing process is thus a major scientific challenge that requires integration of molecular, cellular, genetic, clinical and physiological approaches.

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