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Chapter 2

The Role of Telomere-Telomerase System in Age-Related Fitness Decline, a Tameable Process

Giacinto Libertini
Independent Researcher,
via Cavour 13, Caivano 80023, Naples, Italy
Azienda Sanitaria Locale NA3, giacinto.libertini@tin.it

Abstract

In our body there is a continuous cell turnover. Every day innumerable cells die by programmed cell death, in particular apoptosis, and are replaced by others deriving from stem cells. With the passing of time, this turnover is limited by sophisticated mechanisms, genetically determined and regulated, which control the telomere-telomerase system and therefore cell duplication capacity (replicative senescence) and overall functionality (cell senescence).

Alterations of cell turnover mechanisms cause dramatic syndromes, such as dyskeratosis congenita and Werner syndrome, while the normal age-related slowdown and stopping of this turnover causes a fitness decline that is defined senescence in its more advanced expressions. The fitness decline documented in the wild for many species should not be confused with the mortality increment observed for animals, as *Caenorhabditis elegans* and *Drosophila melanogaster*, in artificial conditions at ages non-existent in the wild.

Many species are not subject to this fitness decline and, in the case their individuals reach very old ages in the wild, are defined as ageless animals or species with ‘negligible senescence’. For some of them, the functionality of the telomere-telomerase system has been documented as unvaried at older ages.

Indeed, the fitness decline appears not an inevitable decay but a very sophisticated function, favoured for its greater inclusive fitness in particular selective conditions, and, being a function, in principle modifiable and governable. This leads to the prospect that senescence will be tamed in the not too distant future, in particular by control of, or more audaciously, by a modification of, the genetic determinants of the telomere-telomerase system. Such a prospect is radically different from the present advances in medical cures that are only increasing the proportion of disabled ultra-octogenarians.

Behind the Scenes: Introduction

When Darwin proposed the hypothesis of evolution by natural selection, two big problems undermined the reliability of the great theory.

The first was the existence of insect species with social organisation (bees, ants, etc.). If natural selection favours individuals with greater fitness and reproductive success, how can one explain the fact that worker individuals of these species nurse the progeny of queens and do not procreate themselves? What possible selective mechanisms can favour the genes determining such odd behaviour? Darwin, the father of modern biology, did not know how to answer: as an improvised patch for a wonderful new dress with a bad tear, he justified all this by maintaining that such behaviour was favoured because it was advantageous for the species [1]. He was wrong, and the correct answer was discovered nearly a century later, as will soon be discussed.

The second problem was even more serious. For many species, *Homo sapiens* included, an increase in chronological age is accompanied by a fitness decline in the wild. That is to say, mortality rates increase with age in the wild [2,3,4,5,6,7,8,9]. Regarding this fitness decline, referred to as “aging” in its more advanced expression (a popular and terrible name), if natural selection favoured the fittest, how was this explicable? Darwin had two alternatives, both difficult and fraught with implications. The first (*nonadaptive hypothesis*) demanded the admission that natural selection was not able to favour genes suited to keeping fitness stable at increasing ages. However, was it possible that natural selection, which is thought to have moulded the eye, brain, hand and numberless marvels in numberless animal, plant and microbe species, failed in the task of keeping fitness stable at greater ages? Moreover, if this is the case, then why has this hypothetical incapacity of selection been greater for some species that age quickly and lesser for other species which age slowly or even not at all?

The other possibility (*adaptive hypothesis*) was that this fitness decline had some unknown evolutionary advantage. This hypothesis seemed even more arduous: how could an anticipated death be evolutionarily advantageous? Who could maintain such a thing without being considered a little muddled, or worse? Darwin could not give an answer to the second problem, thus it was aptly named Darwin’s dilemma [10].

Some years later, August Weissmann, using extraordinary intuition, tried to give an answer. Unfortunately, he did not formulate a clear exposition or give solid scientific proofs, although he did hint that the anticipated death of old individuals was beneficial because this gave more space to new generations which was useful for the evolution of species [11,12]. In short, Weissmann was a supporter of the adaptive hypothesis of fitness decline, although he later disavowed it [12,13]. Furthermore, about the mechanism underlying this decline, he observed that the cells of the various organs and tissues were renewed continuously and that when this turnover slackened or stopped, the organs or tissues reduced or lost their functionality with negative effects on fitness [12].

His adaptive hypothesis was original but not well inferred from a theoretical viewpoint. Moreover, as common experience testifies, all inanimate things deteriorate with the passing of time, so why not assume that living beings, too, are subject to the same inexorable law? In fact, “common sense” seems to strongly suggest that the nonadaptive hypothesis of fitness decline is correct, even if this requires the admission that natural selection is incapable of solving this specific problem.

On the contrary, the cell turnover mechanism hypothesised by Weissmann for the aging was easier to understand and accept but it had an unhappy fortune, too, at least for the next 70 years. Indeed, an illustrious Nobel prize winner, Alexis Carrel, demonstrated that cells explanted and cultivated *in vitro* multiplied an unlimited number of times [14], meaning that Weissmann's hypothesis was groundless and unacceptable.

Poor Weissmann with his intuitions seemed to miss every time!

In 1961 an obscure researcher, Leonard Hayflick, cultivated fibroblasts *in vitro* and discovered that they multiplied a limited number of times, a finding in clear contrast with Carrel's results. After having excluded any factor as a possible cause of such stoppage in cell duplication, he decided to publish his findings. However, the authoritative journal to which the paper was submitted rejected it, with the statement that it was *a priori* unacceptable since the results were in plain contrast with what had been definitively demonstrated and what was accepted as scientifically sound. Fortunately, Hayflick was stubborn and succeeded to publish his paper in a less authoritative journal which was more open to new ideas [15].

Carrel's observations (likely due to errors in cell culture methodology) were overthrown and Weissmann's hypothesis recovered its value! Hayflick stated in 1977 that the limits in cell duplication (Hayflick limit) were the likely cause of the aging: "... if normal animal cells do indeed have only a limited capacity for division in cell culture, the manifestations of aging might very well have an intracellular basis." [16]. However, this statement was in contrast with other ideas that by this time were imposing themselves about the aging [17], i.e.:

> "mutation accumulation" theory — "Aging" is due to the effects of harmful mutations, accumulated over evolutionary time, manifesting themselves at older ages when, in the wild, survivors are few or absent and, consequently, selective forces are too weak to eliminate them [18,19,20,21,22].
>
> "antagonistic pleiotropy" theory — "Senescence" is caused by pleiotropic genes with beneficial effects at early ages and deleterious effects at later ages [23,24].
>
> "disposable soma" theory — "Aging" has environmental or somatic and not genetic causes, and evolutionary responses to them are increasingly limited at older ages by physiological, biochemical or environmental constraints. Therefore, in the evolutionary search of an optimal allocation of metabolic resources between somatic maintenance and reproduction, the second is preferred [25,26].

In a relatively recent paper, Hayflick, as a prophet repudiating himself, did not mention the pivotal importance of cell duplication limits for the mechanisms of aging and instead proclaimed: "Ageing is a stochastic process that ... results from the diminishing energy available to maintain molecular fidelity. This disorder has multiple aetiologies including damage by reactive oxygen species." [27]

In the years 1970-76, I was a simple student of medicine, rich in a wide ignorance and in the foolhardiness of which the ignorant persons enjoy, but —merit among the faults— was strongly struck by Hayflick's results that I considered of extreme importance for aging studies. I became an enthusiast of Hayflick's discovery and of its implications and remained firm in this belief even when Hayflick diminished or denied the importance and the implications of his results.

Stimulated by them, I tried to explain, in precise terms of natural selection, the advantage of a quicker turnover of generations that seemed to me the automatic consequence of the Hayflick limit or, in the case that this limit was unimportant, the advantage of the evident restraints in the individual duration of life for many species, man included, whichever the mechanism causing those restraints was.

Knowing what Weissmann had hinted at, and stimulated by the suggestions of two botanists [28,29], I formulated a mathematical model demonstrating with precision how a quicker generation turnover allowed a quicker diffusion of a favourable gene within a species. In numerical terms, this study modelled the spreading velocity of a gene within a species, the increment by x% of gene advantage or the reduction by an equal x% of the mean duration of individual life had the same effects (Figure 1).

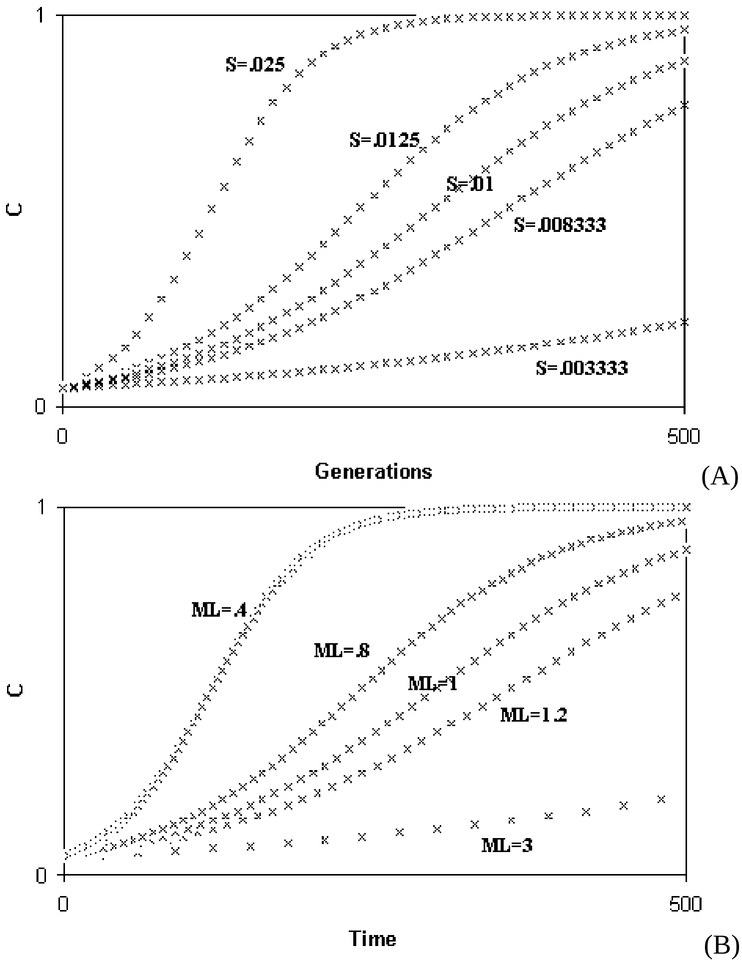


Figure 1. (A) Spreading of a gene C according to the variation of the advantage (S) caused by the gene C, supposing a constant mean duration of life. (B) Spreading of the same gene C according to the variation of the mean duration of life (ML), supposing a constant value of S (= 0.01) [38,39,40].

This study was essentially the reformulation of Weissmann’s intuition in mathematical terms. As examples in more comprehensible terms: populations of bacteria or insects under the action of antibiotics or insecticides become resistant to the action of these substances in the space of a certain number of generations. For a bacterium this time could be a few days, for an insect few years. For a human population, an equivalent evolution would require centuries as a consequence of the much slower turnover of generations.

However, this argument only pointed out that, all other things being equal, species with a smaller mean duration of the life (i.e. a quicker generation turnover) are favoured. This study did not prove that within a species a gene limiting the duration of life would be favoured by natural selection. Darwin’s argument that this could be favoured in terms of group selection was untenable [30,31] and Weissmann’s intuition needed something more.

Then, I obtained a likely solution from the extraordinary results of a group of researchers [32,33,34,35,36], whom in the intervening years had formulated a brilliant solution for the problem of the evolutionary mechanisms explaining the organisation of the social insects, the first of the above said great problems undermining the theory of evolution.

Until this time, accepted evolutionary arguments were only in terms of selection proportionate to the fitness of the single individual (individual fitness). In fact, for a gene causing an advantage or disadvantage (S) and acting in an individual with the capacity P of having progeny (reproductive value), the selective force (F) operating in favour or against the gene was calculated as proportional to the product of S by P:

$$F \propto S \cdot P \qquad (1)$$

In the solution maintained by those researchers, it was pointed out that for a gene existing and acting in an individual, defined as individual 1, it was necessary to also calculate the effects of the actions of the same gene on the fitness of other individuals (2, 3, ..., n) genetically related with the individual 1 (inclusive fitness). Indeed, for each gene C, it was necessary to calculate the sum of the values of the advantage or disadvantage for each individual X for which the gene C had a consequence (S_x), multiplied by the reproductive value of each individual X (P_x), then multiplied by the coefficient of relationship between individual 1 and individual X (r_x, or the probability that gene C is present in X).

Therefore:

$$F \propto \Sigma(S_x \cdot P_x \cdot r_x) \quad \text{with X from 1 to n} \qquad (2)$$

As a simple and easily comprehensible example, a young mother with the act of nursing her child reduces her fitness (because she spends energy resources) but the same act is indispensable for the survival of her child, who has a 50% probability of having the same genes as his mother. Therefore, the small disadvantage for the mother caused by the nursing ($S_1 = -y$, with y having a small value) is clearly exceeded by the great advantage for the child ($S_2 = 1$) reduced by 50% ($r_2 = 0.5$): the inclusive fitness of a gene that determines the nursing is positive and the gene is favoured by natural selection. In numerical terms, supposing that the mother is young and her reproductive value is maximum ($P_1 = 1$) and the child reproductive value is also high ($P_2 >> 0$; supposing that only 60% of the children reach the reproductive age, $P_2 \approx 0.6$):

$$F \propto S_1 \cdot P_1 \cdot r_1 + S_2 \cdot P_2 \cdot r_2 = -y \cdot 1 \cdot 1 + 1 \cdot 0.6 \cdot 0.5 = -y + 0.3 >> 0 \qquad (3)$$

With the simpler formula (1), it is clear that the nursing gene would be eliminated by natural selection.

Other two examples are illustrated in Figure 2.

It has to be noticed that formula (2) becomes formula (1) in cases where the gene only has effects for individual 1, because the coefficient of relationship of individual 1 with himself is clearly 1 ($r_1 = 1$).

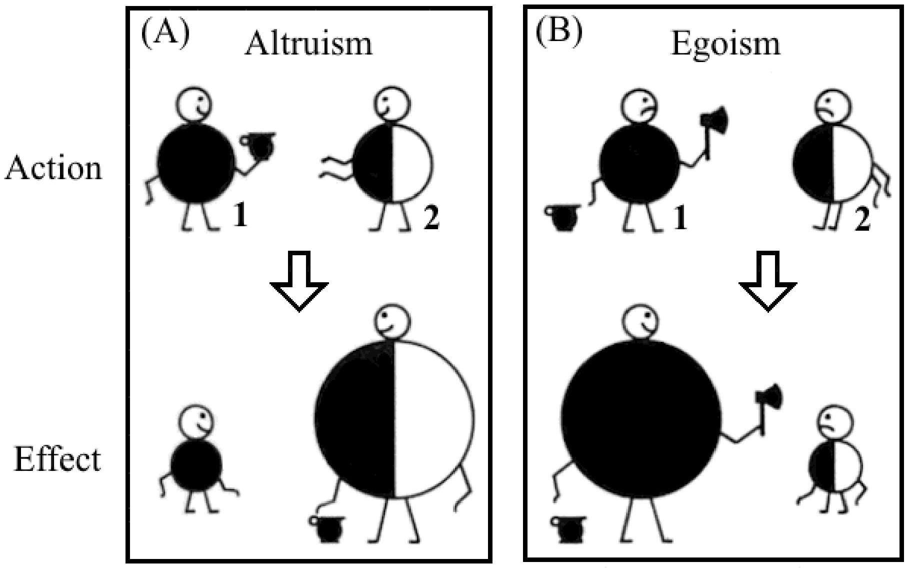


Figure 2. Actions between two brothers (<1> and <2>) that, being brothers, have in common half of the genes (r = 0.5). (A) "Altruism" - By effect of a gene X, <1> gives something of his resources to <2>, increasing the fitness of <2>. His fitness is reduced but the fitness of <2> is increased more than the double of the reduction for <1>: the gene X is favoured by selection. (B) "Egoism" – By effect of a gene X, <1> subtracts something of the resources of <2>. His fitness is increased but the fitness of <2> is reduced less than the half of this increase: the gene X is favoured by selection. The picture is from Wilson, partially redrawn [35].

Following this principle, because for the peculiar genetic mechanism with the difficult name haplodiploidy (males are haploid, while females are diploid) the coefficient of relationship between two sister bees is 0.75 while that between a mother bee and its daughter is 0.5 (see Figure 3-C), the inclusive fitness of a gene determining the nursing of a sister grub by a worker bee is favoured by natural selection more than a gene determining the nursing of a daughter grub by its mother bee. This is the same for ants.

With all the details and quibbles that such a scientific formulation involved, inclusive fitness adequately explained the social organisation of bees and ants. Darwinian theory was enriched and an unresolved problem was settled!

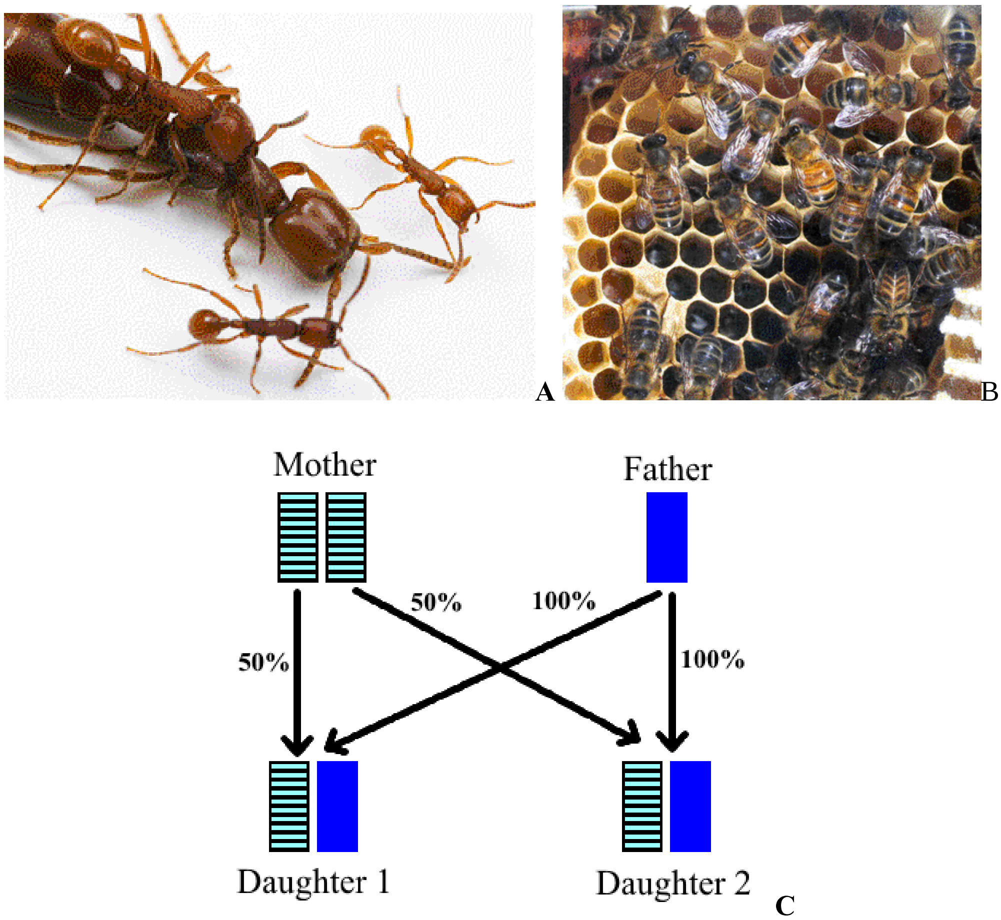


Figure 3. (A) A queen ant with some worker ants; (B) A beehive; (C) For ants and bees, females are diploid while males are haploid. Therefore, a mother gives to a daughter 50% of its genes, while a father gives 100% of its genes. The probability that a gene is the same in the mother and the daughter, alias the coefficient of relationship (r) between mother and daughter, is 0.5. Otherwise, r between the two daughters is 0.75, the arithmetical mean between 50% genes in common with the mother and 100% in common with the father.

Drawing my inspiration from the same concepts, I realised that a life limiting gene was certainly harmful for individual where it acted (individual 1), but, considering that the death of individual 1 gave space for other individuals (2, 3, ... n) and allowed a quicker turnover of generation and so a quicker evolution for 2, 3, ... n, if those individuals were genetically related to individual 1, the inclusive fitness of the gene could be positive and in certain conditions (in short, a population divided in demes and numerically stable, alias K-selection [37]) the gene would be favoured by evolution.

Wonderful, I had a possible solution for Darwin's dilemma, the evolutionary key for the basis of aging!

I had to publish my arguments, but I feared greatly that others could steal my ideas. I thought they would be easily understood, accepted and therefore exploited by others, but how wrong I was!

Consequently, I decided that before the publication of my ideas on a scientific journal, for precaution it was necessary to print a book stating them so that any doubt of first claim would be cancelled.

Therefore, in 1983 I published in Italian a book where the above said ideas, and others, were expounded [38]. I sent copies of the book to many personalities of the Italian scientific world.

Well, the book was a total commercial failure and no one replied (but 21 years later Prof. Pietro Omodeo, an illustrious father of Italian biology, reminded me of the copy I'd sent to him and praised it!). However, my purpose was not economic and I diligently began trying to publish my hypothesis in the form of a scientific paper in an authoritative journal.

The task revealed itself to be very challenging. I had never published a scientific paper, my knowledge of English was rudimentary and my academic background was non-existent. Moreover, internet was not yet born and to even obtain a copy of a scientific paper was difficult for me. It was like trying to climb a Himalayan peak without any experience of mountaineering, with no training, without the help of sherpas and with inadequate and insufficient equipping. My only "force", besides the conviction of the correctness of my hypothesis, was an ignorance of this inexperience combined with a good strong dose of stubbornness. About four years were necessary but in the end, after various rejections and after various modifications and corrections of the paper, I succeeded in publishing my hypothesis in the Journal of Theoretical Biology (which rejected it two times before the acceptance!).

To publish my paper, I had to specify that my topic was not on the imprecise concept of 'aging' or 'senescence', but on the "increasing mortality with increasing chronological age in the wild" (IMICAW) [39], a concept that has later referred to as "actuarial senescence in the wild" [8,9]. In effect, in the wild, the age-related fitness decline, which is a reality for many species, man included, allows only a few individuals to reach that deterioration of all the functions called old age or "state of senility", while in protected conditions (captivity, civilization, etc.) many individuals reach that age (Figure 4).

In the next weeks after publication, I received by post from each part of the world (Siberia, Bulgaria, South Africa, Alaska, Argentina, USA, France, ... even one from Italy ...) 48 requests of reprints of the paper. I satisfied all the requests and for each of them I asked for comments, criticisms and suggestions. I was in very high spirits ... but no one replied.

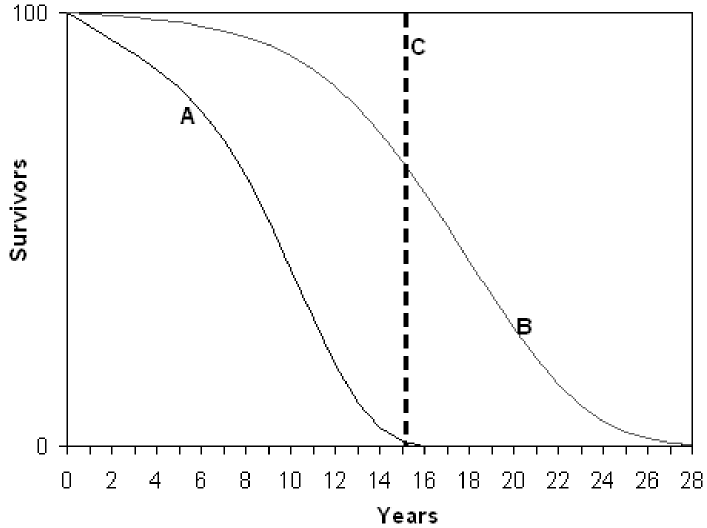


Figure 4. Curve A: Life table in the wild of a species with a progressive fitness decline; Curve B: life table of the same species in artificial conditions of lowered mortality; Line C: arbitrarily defined line marking the beginning of the old age, or "state of senility" (at the time when the reduced fitness in the wild has become smaller than an arbitrarily established value). The fraction of individuals surpassing this line is small in the wild and the grade of their functional decay is in the arbitrarily defined range of the "state of senility". With artificially lowered mortality this fraction becomes appreciable or even preponderant [40].

In the meantime, I sent a copy of the paper to an Italian journal of medical popularisation, along with a note explaining the importance of the topic and my willingness for a short informative article. At once the journal agreed to my proposal and asked me to send them the article and my photograph. Unfortunately, a few days later the journal director called me in person and, being very apologetic, informed me that the article would not be published because their trusty "experts" did not share my hypothesis. My objection that a popularisation journal should spread the news and not do a new judgement by referees, was useless: my hypothesis was too much in contrast with widely diffused and well established ideas.

Moreover, I was quite aware that I had formulated an evolutionary explanation for age-related fitness decline but had not proposed a physiological mechanism by which this decline could occur. I guessed that somehow the Hayflick limit was the key, but the topic was muddled for me and I did not dare to hazard hypotheses.

I thought that the time was not yet right and decided I should neglect any study on the topic for about fifteen years. And so I did, devoting my attention to various other things, things for which a mention here is inappropriate. Only every now and then did I turn my attention to what had once been my obsession for so long.

In 2000, reading in Nature an "authoritative" paper on aging [17], I noticed the mention, as proof of the current gerontological theories, of a paper whose title proclaimed: "Evolutionary Theories of Aging: Confirmation of a Fundamental Prediction, with Implications for the Genetic Basis and Evolution of Life Span" [9]. At once I looked for a copy of the article and then realised with astonishment that the statement of the title was in utter contrast with the results exposed in the text and with the evaluations and conclusions of the same Author! In fact, Ricklefs who was studying the life tables of many mammal and bird species in the wild, had found that the reduction of life span caused by the progressive increment of mortality ("proportion of senescent deaths" in his terminology) was inversely related to the environmental, or extrinsic, mortality (Figure 5). He declared openly that this finding was in plain contrast with the predictions of current gerontological theories! However, in my theory I had predicted that what Ricklefs termed 'the proportion of senescent deaths' should be inversely related to the extrinsic mortality - precisely as was documented by him. In my paper, I had given this paradoxical phenomenon the suggestive name "Methuselah effect" [39], but I had no empirical proofs of this prediction. Well, ten years after the publication of my paper, an authoritative scholar, whilst attempting to confirm predictions of the traditional theories, on the contrary found a clear proof in contrast with them and in support of my hypothesis! But, why was the contrary conclusion proclaimed in the title? I do not know, but I fear that with a title conforming to the facts and conclusions exposed in the text, the paper would have suffered many difficulties in being accepted.

However, I decided that the time was now mature for a renewed attention by myself to the topic of age-related fitness decline in the wild, and to his human terrible outcome in its amplified expression at older ages in protected conditions, namely the senile state.

In the following months and years, I sought for and carefully consulted many papers and books regarding subjects such as telomeres, telomerase, replicative senescence, cell senescence, apoptosis, cell turnover, and diseases caused by cell turnover disorders, which I considered as deeply linked to what I investigated.

I looked for and obtained many internet contacts with scholars of various disciplines.

I wrote to Hayflick too, proclaiming my deep-rooted admiration for his essential discovery and with respectful boldness I asked for his copartnership to what I was elaborating. Hayflick replied with kindness that he did not share my opinions and that for him aging was the consequence of a large set of cell alterations well documented by innumerable experiments. With pride and dismay I perceived that I had become the standard bearer of the implications of Hayflick's discovery - in contrast and beyond the position of the same Hayflick! An enormous weight on weak shoulders, but I was not discouraged ...

Many scholars replied to my requests, some with simple encouragements, and others with useful criticisms or suggestions. In some cases, the lack of sound objections or criticisms was an indirect confirmation of the arguments that I was proposing. However, I must express a particular gratefulness to Jerry Shay, for his useful suggestions on telomeres and telomerase, a topic in which he is an undisputed master; to Eric Le Bourg, Josh Mitteldorf and Theodore Goldsmith for their useful mentions of important papers; to Richard Ricklefs for his attention and kindness. In particular, I am profoundly grateful to Leonard Hayflick for his appreciation of my last paper (2008), which I have accepted as a sort of scientific blessing.

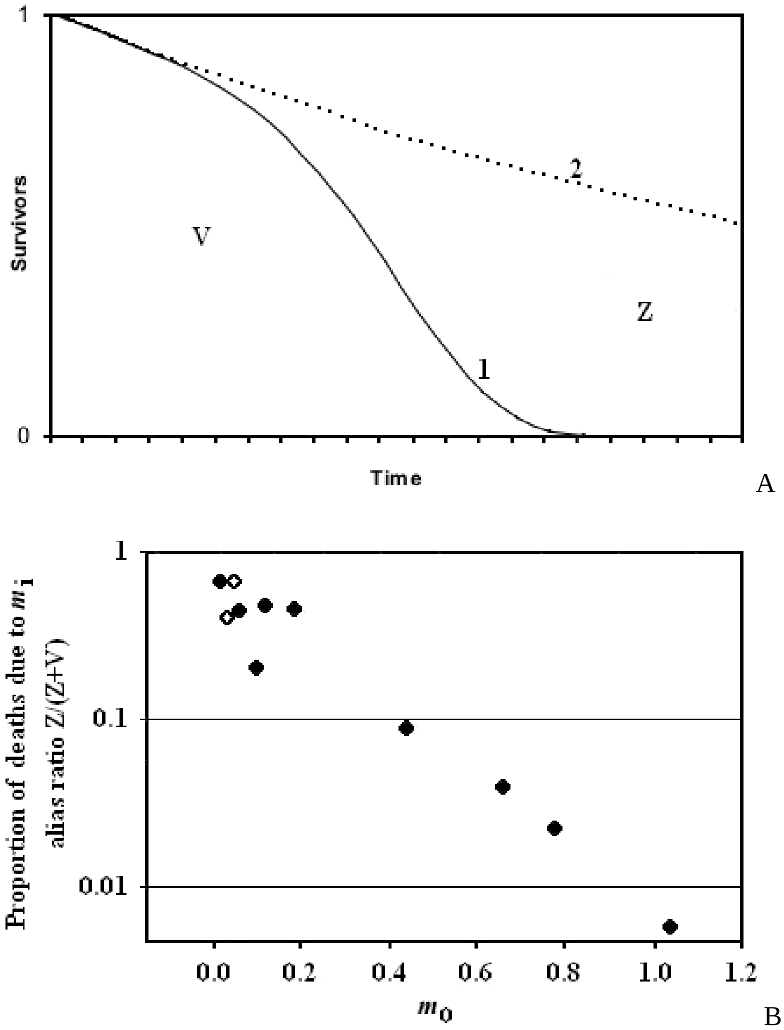


Figure 5. Ricklefs' observation. (A) Life table in the wild of a species with an age-related increasing mortality, determined by extrinsic mortality (m_0) plus intrinsic mortality (m_i) (Curve 1, continuous line); hypothetical life tables with the action of m_0 only (Curve 2, dashed line); V is the area delimited by Curve 1; Z is the area between Curves 1 and 2, alias the "proportion of senescent deaths" (P_s). (B) Inverse significant correlation between m_0 and the proportion of deaths due to m_i (P_s), or ratio Z/(Z+V). Data are from Ricklefs [9], Table 2 (p. 30). Ricklefs' Figure 7 (p. 34) has been redrawn. Ordinates are in logarithmic scale. Open symbols refer to mammal species, solid symbols to bird species.

After some years of study and some useful rejections by qualified journals, in 2006 I published a paper [40] in which my hypothesis was reaffirmed and an overall interpretation of aging, both in terms of evolutionary mechanisms and in terms of underlying physiological mechanisms, was given. Moreover, I clearly expressed the differences between the current ideas about aging and the new proposed paradigm.

In the next months I wrote a further paper, published in February 2008 [41], where the strong empirical evidence in support of the adaptive interpretation of age-related fitness decline in the wild (in different ways formulated by various authors [10,11,39,40,42,43,44]) and against nonadaptive hypothesis [18,19,20,21,22,23,24,25,26] and historical hypothesis [45] was given.

In the same article, I maintained that experiments about the modifications of life tables of animals such as *Caenorhabditis elegans* and *Drosophila melanogaster* were of little importance for the study of aging in species such as ours. The reason for this was because *C. elegans* and *D. melanogaster* reach ages in the laboratory that are non-existent in the wild, thus the studies are observing laboratory artefacts. Furthermore, these animals have no cell turnover, whereas our species do.

The following exposition is a description of how facts well documented by empirical evidence and supported by plausible arguments indicate the mechanisms underlying age-related fitness decline and aging, as I have described in the above said papers, but with further evidence, details and deductions.

Please, a bit of attention, the explanation of an upsetting drama involving emotionally and physically all of us begins!

The Protagonist: Telomere-Telomerase System

Hayflick demonstrated that cultivated human diploid fibroblast-like cells (HDF) from a variety of normal tissues have a finite growth potential *in vitro*, i.e. divide only a finite number of times (“Hayflick limit”) [46,47].

Moreover, it was shown that foetal HDF display a consistently greater number of population doublings (approximately 50) than cells derived from adult tissues (20-30 doublings) [46]. Growth potential of skin HDF from donors of different ages showed a reduction of potential doublings of 0.2 doubling per year of life [48]. A decline in growth potential was reported for epidermal keratinocyte culture [49], arterial smooth-muscle cell [50] and lens epithelial cell [51]. A positive relationship between growth potential of HDF cultures and the maximum life span of the species from which the cells are derived was reported [52].

In 1976, the Hayflick limit was documented *in vivo* too [53].

In 1975, the unknown mechanism limiting the number of duplication was shown to be in the nucleus [54].

However, it was known from 1972 that DNA polymerase could not replicate a whole molecule of DNA and a little part of an end of the molecule would be unreplicated at each duplication [55]. In the same years, Olovnikov hypothesised that the shortening of DNA molecule at each duplication after a certain number of times could block cell replication capacity and that this could explain the Hayflick limit [56].

In 1978, the end of the DNA molecule, defined telomere, which at each cell replication shortens [57], was shown in a protozoan species to be a simple sequence of nucleotides, TTGGGG, repeated many times [58]. Later, for mammals, man included, the repeated sequence was demonstrated to be only a little different (TTAGGG) [59] but common to slime molds, trypanosomes, and other vertebrates and organisms [60]. Its evolutionary conservation, shared even between mammals and unicellular animals, certainly indicated that the structure has great importance.

However, if the Hayflick limit originated from telomere shortening at each replication, an explanation for cells with numberless replications, such as germ line cells, was needed. The discovery of the enzyme telomerase that elongates telomeres was the solution [61], and was confirmed by its presence in immortal human cell lines [62].

This enzyme was shown to be repressed by regulatory proteins [63]. Moreover, telomerase deactivation caused telomere shortening and a reduction of growth potential [64]. Conversely, telomerase activation caused telomere lengthening and cell immortalization [65,66,67,68,69].

Graduality of duplication blockage in a cell population. The simple description that cells with activated telomerase have an unlimited duplication capacity, while cells with inactivated telomerase show a limited duplication capacity strictly proportional to telomere length, was imperfectly supported by empirical data and a more sophisticated and realistic model was suggested in a review by Blackburn [70]. Indeed, if somatic cell growth potential is strictly proportional to telomere length, it would be totally unimpaired up to a critical length, while under this length, namely starting from a certain number of replications, there would be a sudden slump of the growth potential. However, cell populations show a progressive reduction of growth potential starting from early ages, that is, for single cells, even with telomeres having the maximum length, the passage from “cycling state” (duplication possible) to “noncycling state” (duplication impossible) is stochastic [71,72]. In the aforesaid review it was suggested (“Blackburn’s hypothesis”) that telomere can switch stochastically between two states: capped with particular protective nucleoproteins and uncapped. Capping preserves telomere physical integrity, allowing cell division to proceed. Uncapping occurs normally in dividing cells, regardless of telomere length, but the probability of returning to the capped state is proportional to telomere length and the uncapped state, if left uncorrected too long, elicits the passage to the noncycling state (Figure 6).

Imperfect relation between telomere length and Hayflick limit. To contrast the possible objection that species, such as the mouse and hamster, with long telomeres [73] age precociously, it is necessary to point out that Blackburn’s hypothesis does not require, for different species, a fixed ratio between telomere length and the stability of the telomere-capping nucleoproteins complex. It is easily assumable that the stability of the complex and, more generally, the modulation of telomere-telomerase function is different for each species [74].

However, according to this model, and with the support of the aforesaid observations, a cell population with inactivated telomerase and with telomeres initially at their maximum length, shows, from the beginning, a gradual reduction of duplication capacity. This gradual reduction of duplication capacity is at first minimal, but later increases. Besides, even cells with telomerase activated and therefore telomeres always at maximum length, should show a small percentage of cells passing to the noncycling state at each division. Stem cells, unlike germ cells, have levels of telomerase activity that are only partially able to stabilize telomere length [75] and, therefore, *in vivo* could not indefinitely replace the differentiated elements in cell populations that are in renewal [74].

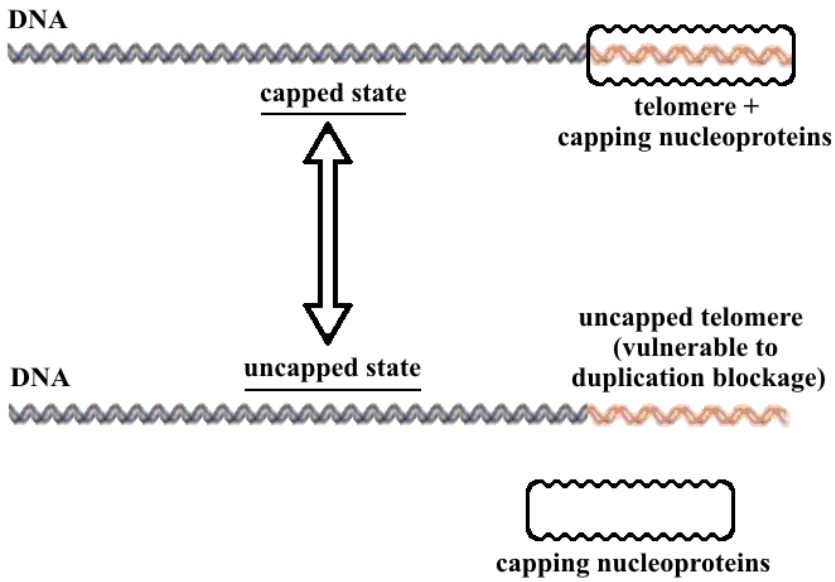


Figure 6. Telomere switches between capped and uncapped states. The probability of being in the uncapped state increases with telomere shortening at each duplication. In the uncapped state, the telomere is seen as a broken end and this can cause an end-to-end joining that stops cell duplication capacity.

Cell senescence. In correlation with the progressive shortening of telomeric DNA, the expression of many genes, among those usually expressed in the cell, becomes impaired, jeopardizing overall cell functionality and, consequently, the functions of extracellular matrix and of other near or physiologically interdependent cells. It has been extensively and soundly documented that this decay of cell functions (cell senescence) and the progressive reduction of cell duplication capacities (replicative senescence), somehow depends on the relative shortening of telomeric DNA (Fossel's "cell senescence limited model") [74].

About the mechanism of this gradual alteration of gene expression: "One model of telomere-gene expression linkage is an altered chromosomal structure (Ferguson et al., 1991), such as a heterochromatin 'hood' that covers the telomere and a variable length of the subtelomeric chromosome (Fossel, 1996; Villeponteau, 1997; Wright et al., 1999). As the telomere shortens, the hood slides further down the chromosome (the heterochromatin hood remains invariant in size and simply moves with the shortening terminus) or the hood shortens (as the telomere is less capable of retaining heterochromatin). In either case, the result is an alteration of transcription from portions of the chromosome immediately adjacent to the telomeric complex, usually causing transcriptional silencing, although the control is doubtless more complex than merely telomere effect through propinquity (Aparicio and Gottschling, 1994; Singer et al., 1998; Stevenson and Gottschling, 1999). These silenced genes may in turn modulate other, more distant genes (or set of genes). There is some direct evidence for such modulation in the subtelomere ..." [74] (Figure 7).

It is likely that the proteinic "hood" and the "capping nucleoproteins" are the same thing because: 1) they act in the same part of the chromosome; 2) telomerase activation causes both telomere lengthening with cell immortalization and the full reversal of cell senescence manifestations [65,66,69].

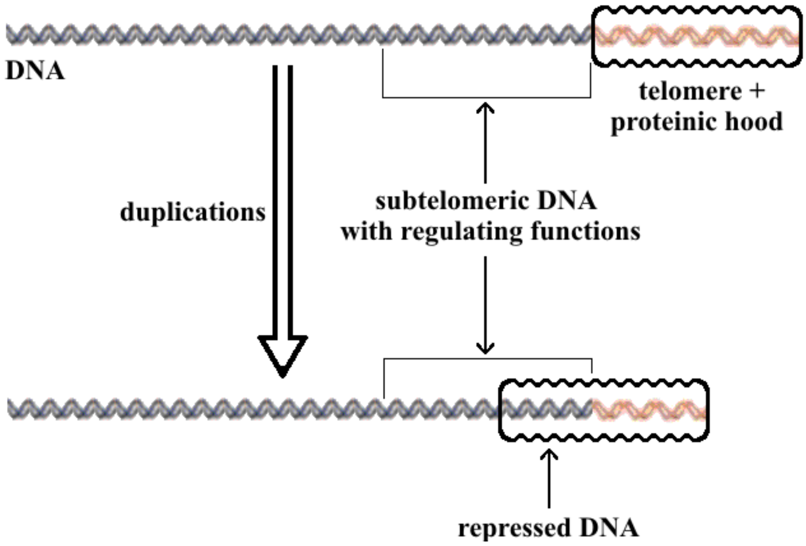


Figure 7. With telomere progressive shortening the expression of many genes results to be impaired. It is likely that near to telomere there is a tract of DNA regulating overall cell functionality and that with telomere shortening the proteinic "hood" alters this regulation [74].

The arrangement and action of subtelomeric DNA, which is of great importance for cell overall functionality, is directly jeopardized by telomere shortening due to its position. This would be evolutionarily illogical and inexplicable if the hypothesis that this "illogicality" contributes to mechanisms favoured by natural selection for determining age-related fitness decay is not accepted.

The reset of telomere clock. Successful fertilization, both in reproduction and in cloned animals, requires the resetting of "telomere clock" [74]. In other words, cells must somehow establish the initial length of the telomeric sequence, since each subsequent shortening of this length will increase the probability of replicative senescence and cell senescence. With a particular mechanism, which is imaginable (Figure 8), a telomere regulates its future functionality without the conditioning of its initial length, whose value is "largely irrelevant" [74]: two *Mus* strains, one with long (20 kb) telomeres and the other with short (10 kb) telomeres have equal life spans and similar progressive alterations in gene expression patterns. The same is true for donor animals and cloned animals that are derived from cells with reduced telomeres [74].

The case of knockout mice. In comparison to humans, mice and other animals have a shorter life span but much longer telomeres [73] and a baseline telomerase activity in most somatic cells [76]. Moreover, in mice with telomerase genetically inactivated (knockout or mTR-/- mice) four [77] to six [78] generations are necessary before the viability and fertility is jeopardized, although dysfunctions in organs with highly proliferative cells are shown in early generations [77,79]. This apparently paradoxical phenomenon is easily explainable with the model illustrated in Figure 8, as expounded in Figure 9.

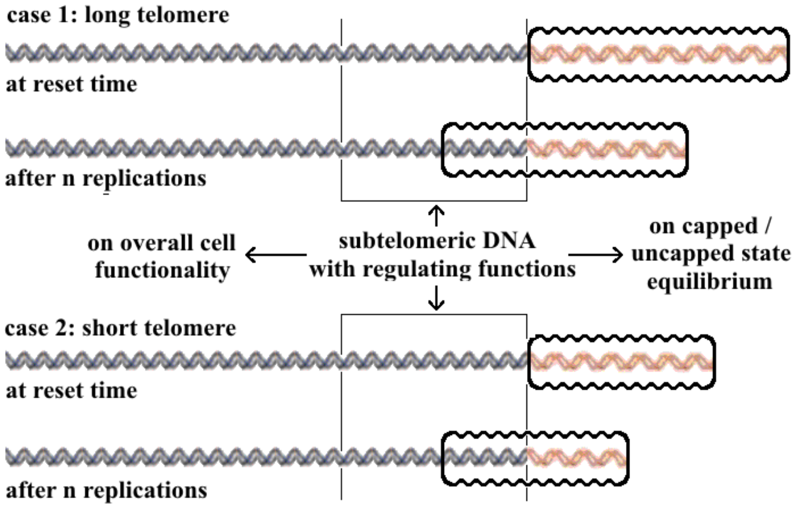


Figure 8. In the resetting of the "telomere clock", the absolute length of the telomere is "largely irrelevant" [74]. During the resetting phase, the length of the proteinic hood could be shaped proportionate to telomere length and remain fixed for all the cell life. If subtelomeric DNA regulates both overall cell functionality (cell senescence) and telomere capped / uncapped state equilibrium (replicative senescence), this could explain the large irrelevance of the initial telomere length for the consequences of its subsequent shortenings. According to this model, the probabilities of replicative senescence and the gradualness of cell senescence after each duplication is proportional to the progressive blockage of subtelomeric DNA and in function of a prearranged pattern, typical of the species and of the cell.

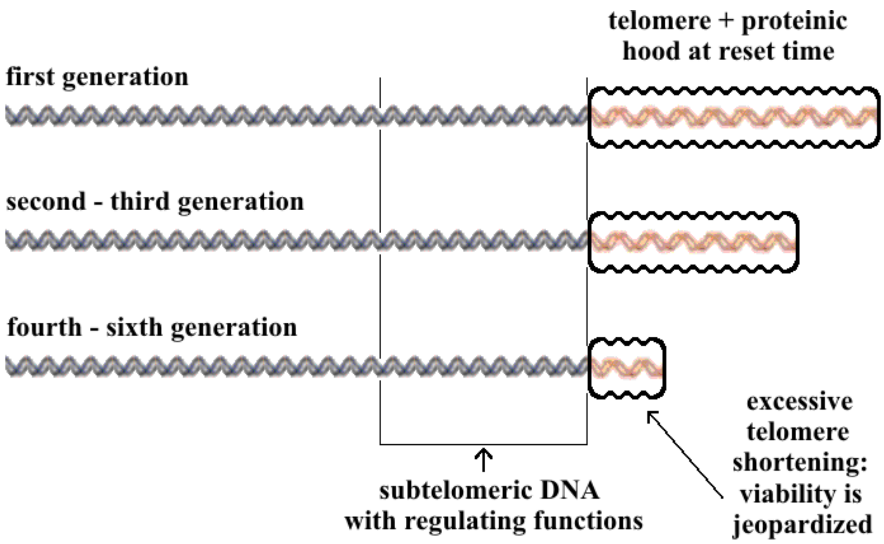


Figure 9. According to the model of Figure 8, in knockout mice, the length of proteinic hood, shaped in the reset phase, is proportionate to telomere length so that telomere functionality is largely irrelevant of its length. The short life span of mice is explained by a low pattern of telomere + proteinic hood complex stability.

With these specifications, the telomere-telomerase system appears to be a highly sophisticated mechanism, genetically determined and regulated, with pivotal importance for cell duplication capacities.

Here, we have a protagonist (telomere-telomerase system). However, we need to know the other protagonist (programmed cell death), the main action (cell turnover), and their cues for the human scene.

The Other Protagonist: Programmed Cell Death

A cell may dies by necrosis because of an accidental event (injury, mechanical stress, infection, ischemia, etc.), or by a form of "programmed cell death" (PCD). The keratinization of an epidermis or hair cell is a form of PCD. Apoptosis is a peculiar form of PCD with an ordinate process of self-destruction with non-damaging disposal of cellular debris that makes it different from necrosis (see Table I). The phenomenon was for the first time described and clearly differentiated from necrosis in the observation of normal liver epatocytes [80] (Figure 10). However, apoptosis is phylogenetically very ancient and is a characteristic of unicellular eukaryote species such as *Saccharomyces cerevisiae* [81, 82].

Selective and programmed cell death by apoptosis is an integral part of multicellular organ development and an important element in lymphocyte interactions and in many pathologic mechanisms. A pivotal function of apoptosis in vertebrates is related to cell turnover in healthy adult organs [83,84,85], as documented for many tissues and organs (i.e., biliary epithelial cells [86]; gliocytes [71]; kidneys [87]; pancreatic β-cells [88]; liver [89]; thyroid [90]; lungs (type II alveolar epithelial cells) [91]; cartilage [92]; prostate [93]; adipocytes [94]; bone [95]; skeletal muscle [96,97]).

Table I. Some differences between necrosis and apoptosis

Necrosis	Apoptosis
Pathologic process caused by non-physiological disturbances (e.g., external injuries, inflammatory factors, lytic viruses, hypothermia, hypoxia, etc.)	Physiologic and tightly regulated process involving activation (e.g., by caspase) and enzymatic steps
Passive process with no energy requirement	Active process energy dependent
Swelling of organelles and of the whole cell; mottled chromatin condensation; random DNA fragmentation	Condensation of cell, organelles and chromatin; non-random DNA fragmentation
Loss of membrane integrity with release of cell's content	Membrane blebbing without loss of integrity
In the end, total cell lysis; the organelles are not functional	Cell falls apart into apoptotic bodies bounded by membranes; the organelles are still functional
Significant inflammatory response	No inflammatory response

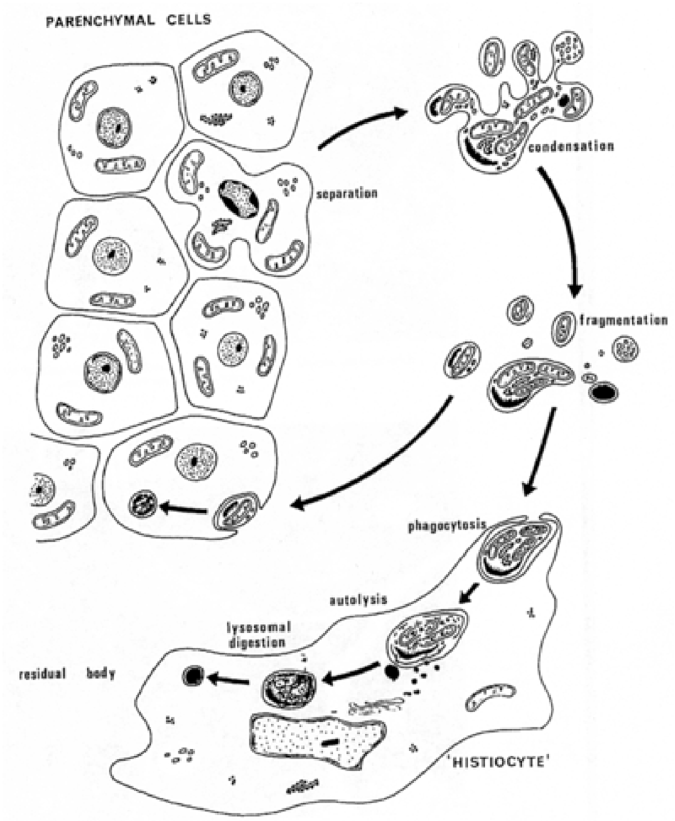


Figure 10. Diagram of apoptosis from the original paper of Kerr et al. [80].

The Main Scene: Cell Turnover

"Each day, approximately 50 to 70 billion cells perish in the average adult because of programmed cell death (PCD).

Cell death in self-renewing tissues, such as the skin, gut, and bone marrow, is necessary to make room for the billions of new cells produced daily. So massive is the flux of cells through our bodies that, in a typical year, each of us will produce and, in parallel, eradicate, a mass of cells equal to almost our entire body weight" [98] (Figure 11).

For many tissues, cell elimination is completed with the removal by macrophages (red cell) or with the detaching from the somatic surface (skin, gut), but for many other tissues and organs, often considered permanent in their cell number, there is a continuous loss of cells by apoptosis. Just in an organ, the liver, apparently stable as cell composition, in an healthy adult subject, apoptosis was described for the first time [80].

The continuous death of cells by PCD is balanced by an equal proliferation of appropriate stem cells, which is regulated and limited by telomere-telomerase system.

Cell turnover is a general pattern in vertebrates, but not for all animals (e.g., the adult stage of the worm *Caenorhabditis elegans* has a fixed number of cells).

Figure 11. A multitude of cells is killed every day by apoptosis. A Colleague commented that this is as: The Slaughter of the Innocents (painting by Beato Angelico).

In short, at least for vertebrates, three categories of cells are currently distinguished:

1. Those with high turnover: e.g., intestinal crypts cells [99];
2. Those with moderate turnover: e.g., cells of the deep layers of skin and endothelial cells [100], heart myocytes ("It remains a general belief that the number of myocytes in the heart is defined at birth and these cells persist throughout life ... But myocytes do not live indefinitely – they have a limited lifespan in humans and rodents. Cell loss and myocyte proliferation are part and parcel of normal homeostasis ... The old heart is characterized by a reduction in cell number and hypertrophy of the remaining myocytes, and this phenotype has been used to argue against the formation of new myocytes. But without cell regeneration the rates of cell death would imply that all myocytes would die during the first few months of a rodent's lifespan. For example, the left ventricle of a young rat contains 13 x 10^6 myocytes, and at any point in time 200 and 93,000 myocytes are dying by apoptosis and necrosis, respectively. Because apoptosis is completed in nearly 4 h and necrosis in roughly 24 h, 94,200 myocytes are lost in one day. Thus, 2.83 x 10^6 cells would die in 1 month, and the total 13 x 10^6 ventricular myocytes would disappear in 5 months." [101]), muscle myocytes (Stem cells from muscles of old rodents divide in culture less than cells from muscles of young rodents [102]; a transplanted muscle suffers ischaemia and complete degeneration and then there is a complete regeneration by action of host myocyte stem cells that is poorer in older animals [103]; there is evidence that apoptosis is a

feature in skeletal muscle fibers in several disease like chronic heart failure or Duchenne muscular dystrophy [104], that reach their deadly end when the replicative capacity of myocyte stem cells is exhausted.

3. Those with no turnover, e.g., neurones, with few possible exceptions [105] but which always have metabolic dependence on gliocytes that are cells with turnover [74].

Atrophic syndrome. In correlation with a significant relative shortening of telomeric DNA, according to Fossel's "cell senescence limited model" [74], a tissue or an organ should show an "atrophic syndrome" with the following features:

a. reduced cell duplication capacity (replicative senescence);
b. reduced number of cells (atrophy);
c. slackened cell turnover;
d. possible substitution of missing specific cells with nonspecific cells;
e. hypertrophy of the remaining specific cells;
f. altered functions of cells with shortened telomeres or definitively in noncycling state (cell senescence);
g. vulnerability to cancer because of dysfunctional telomere-induced instability [106].

A scheme for cell turnover. Cell turnover may be summarized as follows, (though obviously the modulation of this turnover will vary according to the cell type) (Figure 12):

- Stem cells with active telomerase divide themselves and originate somatic cells with replication capacity but with telomerase inactivated;
- Somatic cells with replication capacity but with telomerase inactivated originate differentiated somatic cells with no replication capacity and, after a variable number of duplications and showing a progressive overall function decay (cell senescence), pass from the cycling state to the non-cycling state;
- Somatic cell in non-cycling state (replicative senescence) with increasing cell senescence.

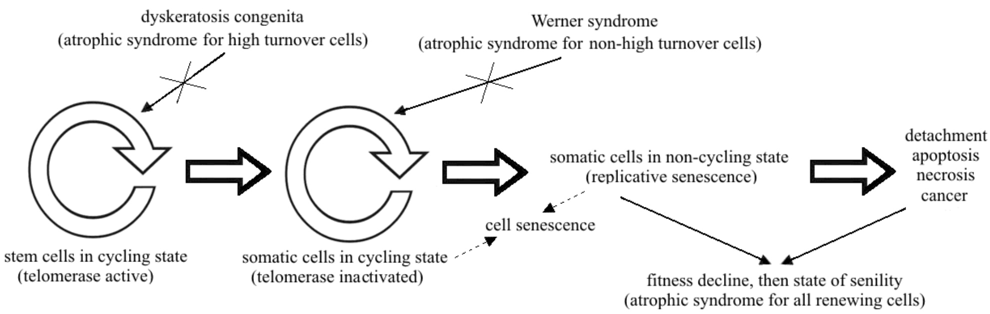


Figure 12. Stem cells with active telomerase divide themselves and originate somatic cells with replicative capacity but with telomerase inactivated. Somatic cells in non-cycling state are originated from these. Replicative senescence and cell senescence contribute to fitness decline that in its more evident expression becomes the senile state.

Two Wrong Performances: Dyskeratosis Congenita and Werner Syndrome

The "atrophic syndrome" with dysfunction of stem cells in the cycling state or of somatic cells in the cycling state should be observable in cells with high and moderate turnover rate, respectively, while in the old age, alias "state of senility" [23], it should be observable in all cells and tissues.

In fact, dyskeratosis congenita, an inherited human disease [107], is an excellent model of the dysfunction of stem cells in the cycling state [100]. Similarly, Werner syndrome is a prototype of the dysfunction of somatic cells in the cycling state, as illustrated in a review [108]. The crucial differences between the two syndromes have been skilfully outlined [100].

Dyskeratosis congenita. An autosomal dominant form of *dyskeratosis congenita* (DC) is caused by mutations in the gene encoding the RNA part of telomerase [109], while with the X-linked form of the disease, levels of telomerase are low and telomeres are shorter than normal [110]. "Problems tend to occur in tissues in which cells multiply rapidly – skin, nails, hair, gut and bone marrow – with death usually occurring as a result of bone-marrow failure." [100]

Defects in DC are present in tissues that have high rates of cell turnover, including those of the blood system and the intestinal crypts, where telomerase activity has been detected [100]:

DC patients also suffer from a higher rate of cancer that can likewise be explained by the lack of telomerase, which results in unstable chromosomes [69,112].

"By contrast, some tissues that have the capacity for cellular replacement, but do not undergo continuous cell turnover, do not express telomerase in their progenitors. It is these tissues – such as the deep layers of the skin or the lining of the blood vessels – that might be expected to suffer most from age-associated telomere depletion, as they have no ability to regenerate telomeres.

Table II. Alterations in dyskeratosis congenital

Organ	Cells expressing telomerase	Defect in dyskeratosis congenita
Hair	Hair follicle	Alopecia
Oral cavity	Squamous epithelium	Leukoplakia
Skin	Basal layer of epidermis	Abnormal pigmentation, nail dystrophy
Lungs	Type 2 alveolar epithelial cells	Fibrosis
Liver	?	Cirrhosis
Intestine	Intestinal crypts	Gut disorders
Testes	Spermatogonia	Hypogonadism
Bone marrow	Progenitor stem cells	Failure to produce blood cells

These tissues would also be greatly affected by defects in other pathways that maintain telomeres, such as DNA-recombination processes. This might explain why Werner syndrome, in which an enzyme involved in DNA processing is affected, yields a closer version of normal (if premature) ageing than does dyskeratosis congenita. In people with dyskeratosis congenita and in telomerase-deficient mice, it is tissues that normally express telomerase that one would predict to suffer most from its loss, and this proves to be the case.” [100]

Werner syndrome (Figure 13). This disease is due to the dysfunction of a member of the RecQ family of helicases [113] causing a dysfunction of somatic cells in the cycling state. In this syndrome, cells show high somatic mutation rates, particularly deletions [114], and a limited replication capacity [48].

Figure 13. A case of Werner syndrome.

Werner syndrome patients show no 'catch-up' growth and a reduced stature, premature greying and thinning of hair, atrophy of skin, regional atrophy of subcutaneous tissue, voice changes (weak, high-pitched), diminished fertility (from the third decade), premature testicular atrophy (from middle age), probably an accelerated loss of primordial ovarian follicles, cataract (from the beginning of the fourth decade), ulcerations around the Achilles' tendons and malleoli, osteoporosis, type 2 diabetes mellitus, a variety of benign and malignant neoplasms, arteriosclerosis, arteriolosclerosis and atherosclerosis, skeletal muscle atrophy, and death usually for myocardial infarction or for cancer [108], but “... no convincing evidence of premature senescence in the central nervous system (CNS)” [108]. The ratio of sarcomas to carcinomas is around 1:1, compared with 1:10 in the general ageing population [115], with origins largely from mesenchymal cells plus some other organ as thyroid, at least in Japanese subjects [108]. Moreover, “... the distribution of the osteoporosis is unusual; the long bones of the lower limbs can be more severely affected than those of the vertebral column. Other unusual radiological features include a characteristic osteosclerosis of the distal phalanges and subcutaneous calcification of the soft tissues. The ulcerations mentioned above are also unusual and can involve the skin around the elbows as well as the ankles.” [108]

All these characteristics may be interpreted as an atrophic syndrome for non-high turnover cells, in consequence of the abnormality in DNA metabolism [108]. In particular: 1) although the crystalline lens has no cell in its core, its functionality depends on lens epithelial cells that show turnover [51]. “Many investigators have emphasized post-translational alterations of long-lived crystalline proteins as the basis for senescent ocular cataracts. It is apparent in Werner syndrome that the cataracts result from alterations in the lens epithelial cells” [108], which is consistent with age-related reduction in growth potential for lens epithelial cell reported for normal human subjects [51]; 2) “Of most interest, however, is the coupling of an abnormality in a RecQ helicase to severe atherosclerosis. In analogy with the skin ulcers seen in Werner subjects, perhaps normal Werner helicase function is required for the efficient repair of the haemodinamic shear stress of arteries. Such repair could be at the level of endothelial cell replication” [108] as suggested for non-Werner subjects [116,117]; 3) Vulnerability to cancer may be explained by telomere shortness and the consequent unstable chromosomes [69,111] and this effect should be manifest for non-high turnover cells such as those of mesenchymal origins and other cells such as those of thyroid; 4) The peculiarities of osteoporosis and ulcerations, osteosclerosis of distal phalanges, subcutaneous calcification of the soft tissues, discontinuities of subcutaneous atrophy may “... reflect an unusual response to repeated, mild local trauma” [108], namely an altered repair capacity due to insufficient duplication capacity of the necessary repair cells; 5) CNS lesions may be secondary to vascular pathology [108] but could be a consequence of neuroglia atrophy [74]; 6) “Skeletal muscle atrophy is at least in part due to disuse, but a primary involvement of that tissue cannot yet be ruled out.” [108]; 7) type 2 diabetes mellitus might be a consequence of β-cell atrophy, as for the same type of diabetes in non-Werner subjects an imbalance between β-cell apoptosis and regeneration rates has been suggested [118,119].

In short, dyskeratosis congenita and Werner syndrome are two model cases of segmental progeria, that is the altered functionality of only a part of cell phenotypes [74]. For example, in Werner syndrome there is no association with Alzheimer disease, commonly observed in the elders. It is plausible that a non-segmental progeria is utterly incompatible with life.

The Main Action: Aging in Man

A simple spontaneous hypothesis about the mechanisms underlying pathophysiological alterations in old vertebrate individuals, namely about “damage resulting from intrinsic living processes” [120] alias “age changes” [27] alias phenomena that are “universal in the species, degenerative, progressive and intrinsic” [121], has been inferred: the more or less precocious aging is the consequence of the less or greater genetically determined cell replication capacity and of the related cell senescence (Fossel’s “cell senescence general model of aging” [74]).

Many experimental data support this hypothesis (e.g., for mice, the p53 tumour suppressor, activated by numerous stressors, induces apoptosis and cell cycle arrest, causing reduced longevity, osteoporosis, generalised organ atrophy and a diminished stress tolerance [122]).

Limiting the argument to the human species because of the large quantity of available data here, if the hypothesis is true, very old individuals, that is those demonstrating “age changes” in their most extreme form - excluding “age-associated diseases” and damages by extrinsic factors (categories 2 and 3, respectively, in Masoro’s 1998 classification [120]) -, should show widespread and pronounced signs of atrophic syndrome for all organs and tissues.

Therefore, we will disregard alterations caused by age-related diseases and, as a matter of prudence, data referred to organs for which hormonal actions are relevant and such as to confuse their analysis (endocrine glands, genital organs, etc.).

Endothelium

The correct functionality of endothelial cells is essential to avoid atherogenesis and its complications, such as cardiac infarctions, cerebral ischemia and other diseases derived from compromised blood circulation [117].

The turnover of these important cells is assured by endothelial progenitor cells, derived from bone marrow, whose number has been shown to be inversely related to age, reduced by cardiovascular risk factors (cigarette smoking, diabetes, hypertension, hypercholesteremia, etc.), and increased by drugs, such as statins, which protect organ integrity [117]. Moreover, with negative relation, the number of endothelial progenitor cells is a predictor of cardiovascular risk equal to or more significant than Framingham risk score [117,123].

In the senile state, diseases deriving from a compromised endothelial function increase exponentially in correlation with the age, even if other cardiovascular risk factors are absent [124]. These factors anticipate and amplify the risk [124], while drugs with organ protection qualities, as statins [125], ACE-inhibitors and sartans [126] counter their effects.

Skin

“Stratum corneal thickness is unchanged in the elderly although its moisture content and cohesiveness are reduced coupled with an increase in renewal time of damaged stratum corneum. ... Human epidermis is highly proliferative but in a steady-state condition dependent, as are other self-renewing structures, on slowly cycling, undifferentiated stem cells. These stem cells are located within the basal compartment of the epidermis – the nonserrated keratinocytes at the tips of the epidermal rete ridges. Loss of rete ridges and consequent flattening of the dermal-epidermal junction is a hallmark of intrinsically aged skin. Such flattening results in a reduction in mean surface area of the dermal-epidermal junction. One study has estimated a reduction in mean area of dermal-epidermal junction/mm^2 from 2.6 at age 21 to 40 years to 1.9 at age 61 to 80 years. These changes are accompanied by a reduction in microvilli – cytoplasmic projections from basal keratinocytes into the dermis. ... The rate of epidermal renewal is reduced in the skin of individuals aged 60 years or greater.

... Melanocytes are decreased in number in intrinsically aged epidermis, although the estimates of this decrease vary from study to study according to the methodologies used to quantitate melanocyte numbers. This said, the reduction is in the order of 8 to 20 percent per decade compared to young adult skin. ... The number of Langerhans cells is reduced in intrinsically aged epidermis, ... Gilchrest et al. demonstrated that subjects aged 62 to 86 years had a 42 percent reduction in the number of Langerhans cells in sun-protected skin as compared to young subjects aged 22 to 26 years. ... Numbers of dermal fibroblasts decrease with age ... Aged skin is relatively hypovascular, particularly due to loss of small capillaries that run perpendicular to the dermal-epidermal junction and form capillary loops. This loss is concomitant with the loss of epidermal rete ridges. Blood vessels within the reticular dermis are reduced in number and their walls are thinned. ... There is an approximate 50 percent reduction in numbers of mast cells in intrinsically aged skin. ... Eccrine glands are reduced in number and function in aged skin. ... Age probably reduces and disorganizes the nerve supply of the skin; indeed there is an approximate two-thirds reduction in numbers of Pacinian and Meissner's corpuscles with age. ... Hair, particularly scalp hair, is lost with age in both sexes. ... Nails grow more slowly in the elderly ... The study of aging skin particularly as a consequence of the ready accessibility of cutaneous tissue is one that presents a paradigm for aging of other organs.” [127]

Eyes

“Atrophy of the fascial planes within the eyelids may lead to herniation of the orbital fat into the lid tissue, producing the 'bags under the eyes' frequently seen in the elderly. Atrophy or disinsertion of the aponeurosis of the levator palpebrae muscle, which ordinarily supports the upper eyelid, may cause the opened lid to fail to uncover the pupil, as seen in senile ptosis, despite normal levator muscle function ... Secretory function of the lacrimal glands declines with age ...” [128]

For crystalline lens and photoreceptor cells, see in the next paragraph.

Orofacial Tissues and Organs

“Structural changes in human oral epithelia with aging include thinning of the epithelial cell layers (e. g., thinning of the lingual epithelial,) diminished keratinization, and simplification of epithelial structure. ... Histologic studies of aging salivary glands show a gradual loss of acinar elements, a relative increase in the proportion of ductal elements, an increase in inflammatory infiltrates, and an increase in fibrofatty tissue.” [129]

“The number of taste buds decreases after age 45, resulting in a decrease in taste sensation...” [130]

Gastrointestinal System

In people over 60, there is an increased prevalence of atrophic gastritis [131].

“Several histologic changes have been demonstrated in the colon, including atrophy of the muscolaris propria with an increase in the amount of fibrosis and elastin,...” [132]

"Using postmortem material, Chacko *et al.* (1969) found that in an Indian population the shape of villi changed on aging. The youngest subjects had finger-shaped villi, but the frequency of broad villi and convolutions increased in specimens from older people. Webster and Leeming (1975a) described similar changes when fresh jejunal specimens from geriatric patients were compared with normal young controls. They found that in the elderly broader villi were more common, and in addition the villi were significantly shorter. ... Andrew and Andrew (1957) noticed an increase in the amount of fibrous tissue between the crypts of Lieberkuhn and a general reduction of cellularity in older mice. ... Lesher, Fry and Kohn (1961), Lesher and Sacher (1968) and Fry, Lesher and Kohn (1961), using autoradiography and tritiated thymidine, showed a prolonged generation time for duodenal crypt cells in old animals and an increased cell transit time (for cells to progress from the crypts to villous tips). In conclusion, the possible expected age changes in the small bowel of man are an increase in broad villi, with a reduction in villous height. These changes may be due to reduced cell production." [133]

Four to six stem cells for each crypt allow the turnover of the absorptive epithelium of small intestine [134].

Liver

Liver volume declines with age [135], both in absolute values and in proportion to body weight [136], and this reduction has been estimated to be about 37 percent between ages 24 and 91 [135]. Liver blood flow also declines with age, by about 53 percent between ages 24 and 91 [135]. However, while liver size declines with age, hepatocytes increase in size, unlike in the liver atrophy that accompanies starvation [137].

Cirrhosis is the final stage of chronic destruction of hepatocytes caused by hepatitis, alcoholism or other factors. When hepatocyte stem cells exhaust their duplication capacities, the liver is transformed by a general atrophic process, often complicated by carcinomas caused by dysfunctional telomere-induced instability [106,111].

Diabetes

Diabetes frequency increases from youth to old age [138]. Pancreatic β-cells show turnover [88] and it has been suggested that type 2 diabetes is caused by insufficient substitution of β-cells killed by metabolic stress [118,119]. In Werner syndrome, diabetes could be caused by impaired replicative process of β-cell stem cells with an insufficient replacement of apoptotic β-cells. In normal old individuals, the progressive exhaustion of β-cell turnover could justify the age-related progressive frequency of the disease.

Drugs effective in "organ protection", as ACE-inhibitors and sartans and statins, reduce the risk of diabetes [139,140].

Heart

In the old heart there is a global loss of myocytes, with a progressive increase in myocyte cell volume per nucleus [141]. "With aging, there is also a progressive reduction in the number of pacemaker cells in the sinus node, with 10 percent of the number of cells present at age 20 remaining at age 75. ... Age-associated left ventricular hypertrophy is caused by an increase in the volume but not in the number of cardiac myocytes. Fibroblasts undergo hyperplasia, and collagen is deposited in the myocardial interstitium." [142]

The decline of cardiac contractile capacities causes an enlargement of the heart that conceals the underlying atrophy of the contractile cells.

"... some increase in the amount of fibrous tissue and fat in the atrial myocardium with a decrease in the number of muscle fibres, and loss of fibres in the bifurcating main bundle of His and at the junction of the main bundle and its left fascicles, with lesser degrees of loss in the distal bundle branches." [143]

Drugs effective in "organ protection", as ACE-inhibitors, sartans and statins, are effective in the prevention of atrial fibrillation [144,145].

Lungs

Lung volumes (FEV1, FVC) decline with age [146]. "The most important age-related change in the large airways is a reduction in the number of glandular epithelial cells ... the area of the alveoli falls and the alveoli and alveoli ducts enlarge. Function residual capacity, residual volume, and compliance increase. ..." [147] (Figure 14).

Statin use reduces decline in lung function [148], justified as due to anti-inflammatory and antioxidant properties [148], but that could be the consequence of effects on type II alveolar epithelial cells, analogous to those on endothelial cells [117].

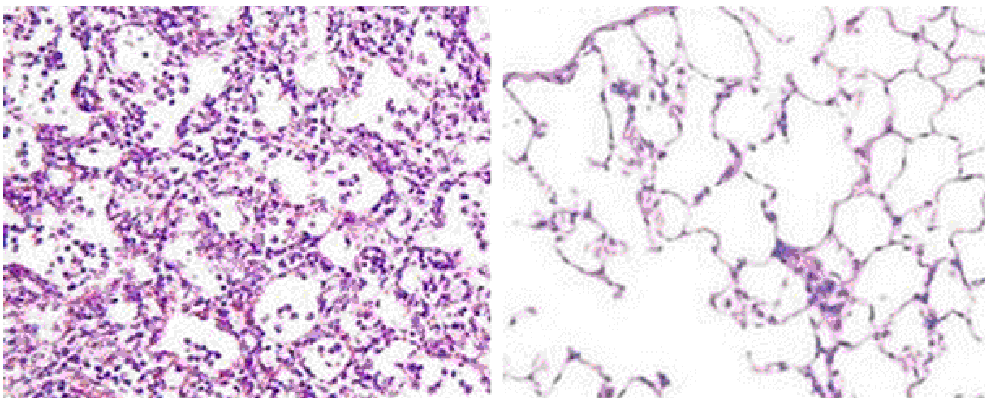


Figure 14. Normal lung (left) in comparison with a lung affected by marked emphysema (right).

Kidneys

“Age-induced renal changes are manifested macroscopically by a reduction in weight of the kidney and a loss of parenchymal mass. According to Oliver, the average combined weight of the kidneys in different age groups is as follows: 60 years, 250 g; 70 years, 230 g; 80 years, 190 g. The decrease in weight of the kidneys corresponds to a general decrease in the size and weight of all organs. Microscopically, the most impressive changes are reductions in the number and size of nephrons. Loss of parenchymal mass leads to a widening of the interstitial spaces between the tubules. There is also an increase in the interstitial connective tissue with age. The total number of identifiable glomeruli falls with age, roughly in accord with the changes in renal weight.” [149]

Microalbuminuria, a simple marker of nephropathy, is “predictive, independently of traditional risk factors, of all-cause and cardiovascular mortality and CVD events within groups of patients with diabetes or hypertension, and in the general population ... It may ... signify systemic endothelial dysfunction that predisposes to future cardiovascular events” [150], and this implicates that drugs effective in “organ protection” defend renal functionality too.

Skeletal Muscle

There is positive correlation between age and muscle atrophy, both in terms of overall muscle bulk and of the size of individual fibers [151,152].

“These changes are to some extent dependent on the fallout of anterior horn cells that occurs with age, but this does not completely explain the process of aging atrophy. In detailed studies it has been shown that the progressive reduction that occurs in muscle volume with aging can be detected from age 25 years and that up to 10 percent of muscle volume is lost by age 50 years. Thereafter the rate of muscle volume atrophy increases, so that by 80 years almost half the muscle has wasted. ... Both reduction in fiber number and fiber size are implicated in the loss of muscle volume.” [153]

In Duchenne muscular dystrophy, there is a chronic destruction of myocytes that are continually replaced by the action of stem cells until these are exhausted [104].

Bone

“Once middle age is reached, the total amount of calcium in the skeleton (i.e., bone mass) starts to decline with age ... This is associated with changes in skeletal structure, resulting in it becoming weaker and more prone to sustaining fractures. For example, the bony cortex becomes thinner due to expansion of the inner medullary cavity, the trabecular network disintegrates, and there is an accumulation of microfractures. ... Bone loss in the elderly is largely a result of excess osteoclast activity, which causes both an expansion in the total number of remodelling sites and an increase in the amount of bone resorbed per individual site. .... Bone loss in the elderly is also thought to involve an age-related decline in the recruitment and synthetic capacity of osteoblasts” [154] (Figure 15).

"Involutional bone loss ... starts between the ages of 35 and 40 in both sexes, but in women there is an acceleration of bone loss in the decade after menopause. Overall, women lose 35 to 50 percent of trabecular and 25 to 30 percent of cortical bone mass with advancing age, whereas men lose 15 to 45 percent of trabecular and 5 to 15 percent of cortical bone. ... Bone loss starts between the ages of 35 and 40 years in both sexes, possibly related to impaired new bone formation, due to declining osteoblast function." [155]

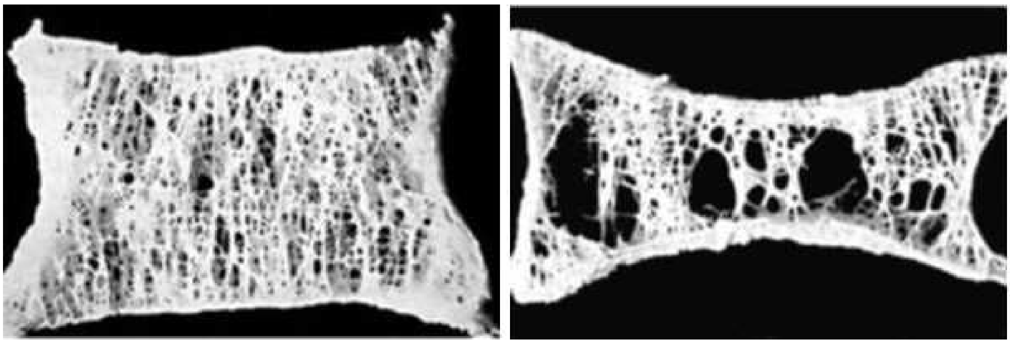


Figure 15. A normal vertebra (left) in comparison with an osteoporotic vertebra (right). The bones of elders are sometimes described wrongly as worn-out while they are clearly atrophic.

Blood

"... red cell indexes are well preserved even in centenarians. ... peripheral blood lymphocyte populations do seem to show a significant change in age, with a fall in total numbers. $CD4^+$ T-helper cells, responsible for major histocompatibility complex class II restricted recognition of foreign antigen and subsequent activation of $CD8^+$ T-suppressor, B-lymphocyte, and granulocyte effector cells of the immune response, show an overall decline with age accompanied by a reduction in capacity to produce virgin $CD4^+$ CD45RA T cells. ... Gradual involution of red marrow continues but is especially marked after the age of 70 years when iliac crest marrow cellularity is reduced to about 30 percent of that found in young adults." [156]

In vitro neutrophil functions (e. g: endothelial adherence, migration and phagocytosis capacity, granule secretory behavior, etc.) are insignificantly affected by age but *in vivo* significantly fewer neutrophils arrive at the skin abrasion sites studied in older people [157]. The proliferative capacity of T lymphocytes to nonspecific mitogens is greatly reduced with aging [158].

It has been suggested that age-related functional decline in adult tissue hematopoietic stem cells limits longevity in mammals [159].

Brain

"...brain weight, on average, remains fairly constant up until 60 years of age, after which a gradual decline sets in leading to an eventual loss of only some 5 percent of the original weight (60 to 70 g) by the ninth decade. ... progressive decline in nerve cell number with aging in areas such as the temporal cortex (middle and inferior temporal gyri), the pre- and post-central gyrus, the striate cortex, and the inferior and superior frontal cortex, leading to average overall losses in old age ranging from about 10 to 50 percent with the greatest changes occurring in the frontal and temporal cortex." [160].

Neurones have no turnover but their survival depends on other cells with turnover, in particular endothelial cells of cerebral arteries and gliocytes [74] (see next paragraph).

Cancer

A thorough review [106] illustrates the well documented hypothesis of telomere dysfunction as an important cause of cancer in old age, especially for cells with higher turnover that are for the most part epithelial.

Cells / Tissues with No Turnover

Crystalline Lens

See discussion about Werner syndrome, argument 1. Besides, statin use lowers risk of nuclear cataract, the most common type of age-related cataract [161]. This has been attributed to "putative antioxidant properties" [161] but could be the consequence of effects on lens epithelial cells analogous to those on endothelial cells [117].

Photoreceptor Cells (Cones and Rods)

Retina cones and rods are highly differentiated nervous cells with no turnover. The top of these cells leans on retina pigmented cells, highly differentiated gliocytes with a turnover rate that declines with age (Figure 16). Each day 10% of the membrane on which photopsin molecules lie are phagocytized by retina pigmented cells and substituted by an equal quantity of new membrane. Each retina pigmented cell serves 50 cones or rods and, therefore, each day a cell metabolises photopsin molecules of about 5 cones or rods, demonstrating a very high metabolic activity. Without the macrophagic activity of retina pigmented cells, photoreceptor cells cannot survive. Replicative senescence and cell senescence of retina pigmented cells limit or stop the functionality of retina cones and rods and then cause their death, i.e. age-related retina macular degeneration (AMD) [162].

With particular deficiencies of retina pigmented cells, AMD arises at lower ages and is considered a specific disease while at later ages its frequency increases exponentially and is considered a feature of the senile state.

Indeed, AMD affects 5%, 10% and 20%, respectively of subjects 60, 70 and 80 years old [163] and it is likely that a large proportion of centenarians suffer from AMD.

Smoking, diabetes, and obesity are risk factors for AMD [164].

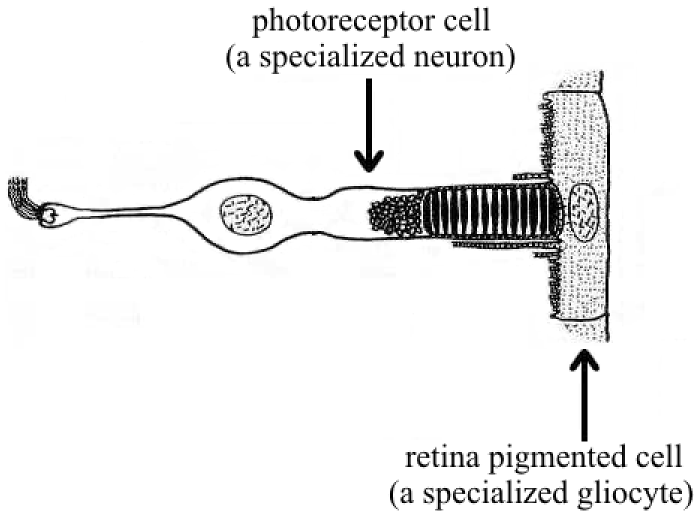


Figure 16. A scheme of a photoreceptor and of a retina pigmented cell.

Neurons

As photoreceptor cells, specialized types of neurons with no turnover, depend on other cells that are specialized types of gliocytes with turnover, perhaps other types of neurons depend from other types of gliocytes.

If this it true, replicative senescence and cell senescence of these gliocytes should cause pathologies similar to AMD. Without the key example of AMD, it has been already hypothesised that Alzheimer disease is dependent from the decline of the function of particular gliocytes (microglia cells) because of the failure of the telomere-telomerase system [74]: "One function of the microglia (Vekrellis et al., 2000) is degradation of β-amyloid through insulin-degrading enzyme (IDE), a function known to falter in Alzheimer disease (Bertram et al., 2000" (p. 233), "telomere lengths of circulating monocytes can serve as an independent predictor in at least vascular dementia (von Zglinicki et al., 2000b)" (p. 235), "A cell senescence model might explain Alzheimer dementia without primary vascular involvement." (p. 235). As for AMD, there are precocious familial cases of Alzheimers, considered as distinct diseases with distinct genetic causes [74], and Alzheimer frequency increases exponentially with age: 1,5% at age 65 years and 30% at 80 [165], with a very high probability that a centenarians is affected by it. There is also an association between Alzheimer disease and cardiovascular factors [166]. Drugs with organ protection qualities such as statins, ACE-inhibitors and sartans, are effective against Alzheimer disease too [167].

Discarding the simplistic deduction that Alzheimer disease is only a consequence of vascular dysfunction, it is likely that there is a common pathogenetic mechanism: endothelial dysfunction caused by low endothelial progenitor cells in the first case, and microglia dysfunction caused by low microglia progenitor cells in the second case. In both cases the telomere-telomerase system is the primary causal factor and cardiovascular / Alzheimer risk factors accelerate telomere failure, whereas "protective" drugs counter these effects (see Figure 17).

A General Scheme

Factors that, for the turnover of each cell type, increase and reduce apoptosis rates should accelerate and slacken, respectively, the physiological age-related decline in turnover and, therefore, the onset of the related function decline (Figure 17 and Table III).

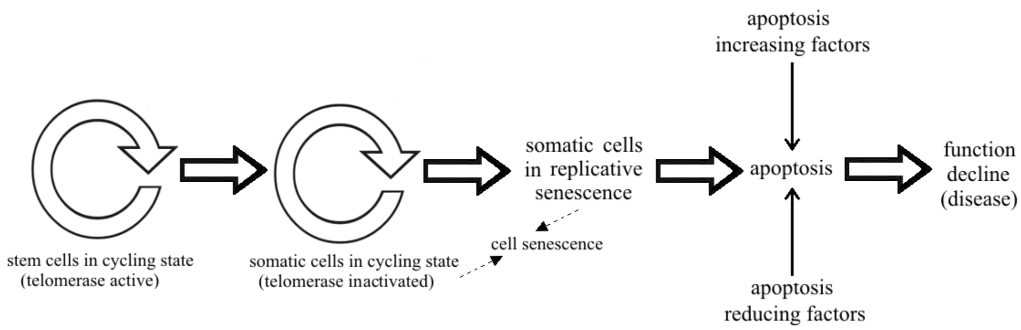


Figure 17. A general scheme for the onset of various diseases. For example, apoptotic rate of endothelial cells is influenced by age as well as changes in the relative risk factors. It is the precise combination of these factors which determines the timing of vascular disease onset.

Table III. Abbreviations: WS = Werner syndrome; DC = dyskeratosis congenita; → X = causing X or accelerating its onset

STEM CELLS OF ...	ALTERATIONS IN THE ELDERS	APOPTOSIS INCREASING FACTORS AND THEIR EFFECTS
Alveolar type II cells	Emphysema	Smoking, chronic inhalation of noxious substances, chronic bronchitis (→ emphysema); DC (→ fibrosis)
Cardiac myocytes	Cardiac insufficiency	Myocarditis (→ dilatative cardiomyopathy)
Endothelial cells	Atherosclerosis	Smoking, hypertension, hypercholesteremia, diabetes; WS (→ atherosclerosis)
Epidermis cells	Skin atrophy	Excessive sun exposure (→ photoaging); DC (→ abnormal pigmentation, nail dystrophy); WS (→ skin atrophy)
Glomerular cells	Renal insufficiency	The same as for endothelial cells (→ renal insufficiency)
Hepatocytes	Hepatic atrophy	Chronic hepatitis, alcoholism; DC (→ cirrhosis, hepatic carcinoma)
Intestinal cells	Intestinal atrophy	DC (→ gut disorders)
Lens epithelial cells	Cataract	Exposure to radiations; WS (→ cataract)
Microglia cells	Alzheimer disease	The same as for endothelial cells (→ Alzheimer disease)
Myocytes	Muscle atrophy	Specific genetic defects (→ muscular dystrophies); WS (→ muscle atrophy)
Osteoblasts	Osteoporosis	WS (→ osteoporosis)
Pancreatic β-cells	Latent or mild diabetes	Hyperalimentation, specific viral infections; WS (→ diabetes)
Retina pigmented cells	AMD	Smoking, obesity, diabetes (→ AMD)

A Badly Interpreted Cue: Telomere-Telomerase System as Oncogenic Factor

If an age-related fitness decline is adaptive, then the existence of sophisticated mechanisms causing this decline, namely the telomere-telomerase system, is indispensable.

Conversely, if an age-related fitness decline is nonadaptive, the telomere-telomerase system needs a plausible and detailed evolutionary explanation for its existence [41].

A speculative justification for the effects (replicative senescence and cell senescence) of the above said system is that of a general defense against the threat of malignant tumors [168,169], in a sort of evolutionary trade-off between aging and cancer restriction [170]. However, this hypothesis does not justify the great differences in duplication limits and overall cell functionality decay from species to species, unless the risk of malignant tumors is postulated as varying from species to species in direct correlation with the limits imposed to cell duplication capacities and to overall cell functionality by the genetic modulation of telomere-telomerase system.

Moreover, there are a number of other problems with the hypothesis that the telomere-telomerase system is a defense against cancer:

1) Lobsters and old rainbow trout, “animals with negligible senescence”, have, in the wild, the same levels of telomerase activity as young individuals [171,172] and increasing problems of carcinogenesis at older ages are not plausible for them because, as their definition states, their mortality rates do not increase with age [41]. For these animals, telomerase action involves no evident oncogenic risk and, therefore the idea that telomerase has an oncogenic effect is implausible in these species.
2) The decline of duplication capacities and of overall cell functionality weakens immune system efficiency [74], which has, for a long time, been known to be inversely related to cancer incidence [173];
3) When telomeres are shortened, there is a great vulnerability to cancer because of dysfunctional telomere-induced instability [106,111];
4) “The role of the telomere in chromosomal stability (Blagosklonny, 2001; Campisi et al., 2001; Hackett et al., 2001) argues that telomerase protects against carcinogenesis (Chang et al., 2001; Gisselsson et al., 2001), especially early in carcinogenesis when genetic stability is critical (Elmore and Holt, 2000; Kim and Hruszkewycz, 2001; Rudolph et al., 2001), as well as protecting against aneuploidy and secondary speciation (Pathak et al., 2002). The role of telomerase depends on the stage of malignancy as well as cofactors (Oshmura et al., 2000); expression is late and permissive, not causal (Seger et al., 2002).” [74];
5) The telomere-telomerase system of yeast (*Saccharomyces cerevisiae*), a unicellular organism, is well studied. Individuals of this species stop their replications after 25-35 duplications [174] that is they show replicative senescence and cell senescence, although not caused by telomere shortening but by another unknown mechanism related to the number of duplications [175]. A senescent yeast cell ends its life with

apoptosis [176] and apoptosis is also triggered in difficult conditions [82]. In both cases, apoptosis divides the cell into metabolically active parts that are usefully and easily phagocytized by other yeast cells. This is done in an orderly way and such behavior has been plausibly interpreted as adaptive [43,44,177,178,179,180]. However, in 1988 it was hypothesized that life limiting mechanisms should be favored in conditions of K-selection, namely: a) with a population numerically constant, as a consequence of a limited living-space, so that only when an individual dies there is place for a new individual; b) with dead individuals replaced prevalently by kin individuals [39]. Colonies of kin yeast cells in a saturated habitat are in these conditions and, therefore, empirical evidence for yeast is a confirmation of this theoretical prediction. In yeast, apoptosis, replicative senescence and cell senescence, determined by genes killing individuals where they act (= negative individual fitness) are shaped by natural selection with clear adaptive aims (= positive inclusive, or - however – supraindividual, fitness) and it is inexplicable that while these phenomena are accepted as adaptive for unicellular species, the same explanation is not considered possible for multicellular species [17]. Finally, given that yeast is a unicellular organism, the telomere-telomerase system and its actions in this species cannot have any value against cancer: the oncogenic risk is non-existent.

In short, the telomere-telomerase system is hardly justifiable as a defense against cancer risk and, lacking other explanations, only the adaptive hypothesis of age-related fitness decline appears a rational cause for its existence.

An Equivocal Cue: The Confusion between Age-Related Fitness Decline in the Wild and Mortality Increase in Laboratory Conditions

In figures 18-A1 and 18-A2, are the life tables of wild species such as the lion (*Panthera leo*) and hippopotamus (*Hippopothamus amphibius*), which demonstrate an age-related mortality increment in natural conditions. These life tables are examples among very many species, our species included, demonstrating the same phenomenon. For brevity, this “increment of mortality with increasing chronological age in the wild” has been called IMICAW [39].

In figures 18-B1 and 18-B2, are the life tables of animals in laboratory conditions, such as the nematode *Caenorhabditis elegans* [181] and the fly *Drosophila melanogaster* [182], which in artificial conditions demonstrate an age-related mortality increment. These life tables are examples among very many species, most of insects included, demonstrating an analogous age-related mortality increment in artificial protected conditions. For brevity, this “increment of mortality with increasing chronological age in captivity” has been called IMICAC [39].

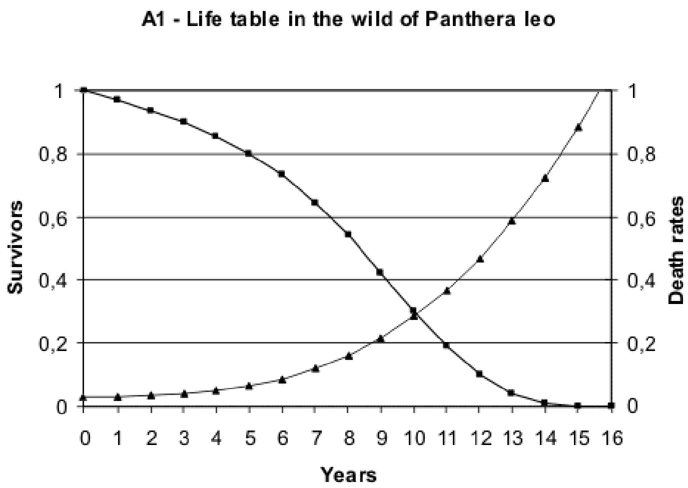
A superficial observer could suppose that IMICAW and IMICAC are the same phenomenon and therefore studies more easily done on the worm or on the fly could explain what happens in species such as the lion, hippopotamus and man.

However, a well-informed observer knows that the ages in which mortality increment starts in laboratory for the worm and for the fly, do not exist in the wild. In fact, the longevity of *Caenorhabditis elegans* "under more natural conditions is reduced up to 10 fold compared with standard laboratory culture conditions" [183] and few individuals of this species remain fertile in the wild after 10 days [184]. Similarly, *Drosophila melanogaster* has a reported adult life span in the wild of 10-12 days [181].

For both these animals, the age-related increasing mortality described in figures 18-B1 and 18-B2 starts at ages non-existent in the wild, meaning that it is no more than a laboratory artefact.

Well, if IMICAW exists by definition in the wild and therefore by definition is influenced by natural selection, while on the contrary IMICAC is non-existent in the wild and therefore is not influenced by natural selection, we should have strong doubts about the conclusions of experiments about IMICAC applied to IMICAW. However, there is another essential difference.

The worm and the fly (and in general the adult insects) are composed of cells with no turnover [181,185], while lion, hippopotamus and man have cells and tissues with turnover. If, as it seems probable, the slowdown and later the stopping of cell turnover, and the correlated cell senescence, are pivotal elements in the fitness decline of animals such as the lion, hippopotamus and our species, it is rather dubious to use experiments on animals with no cell turnover to explain the fitness decline in animals with cell turnover. This is a basic problem, certainly of extreme weight for those interested in the explanation of aging mechanisms. However, in renowned texts on the topic, the problem is not considered [24], and it is frequent that, in very influential journals, experiments modifying the life table of our dear worm or of our beloved fly are presented as meaningful advances in the understanding of human aging [186,187,188]!



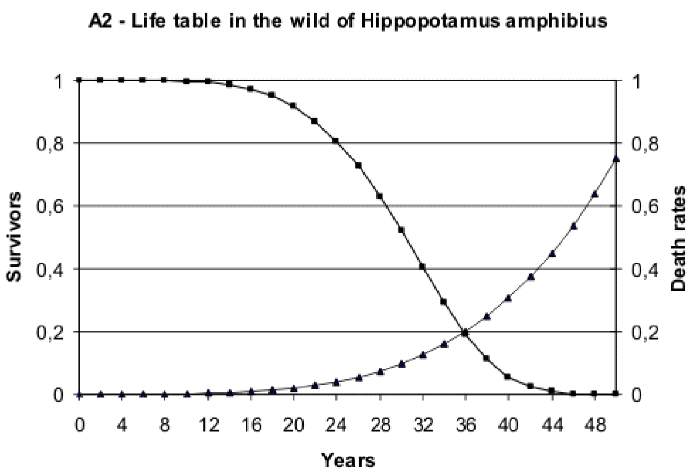
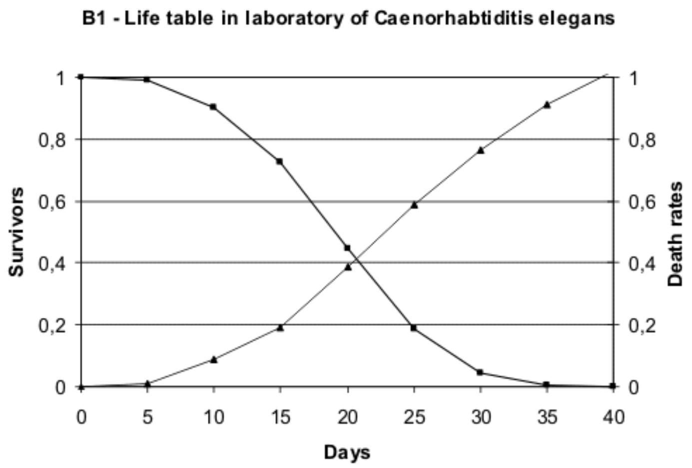


Figure 18. (Continued).



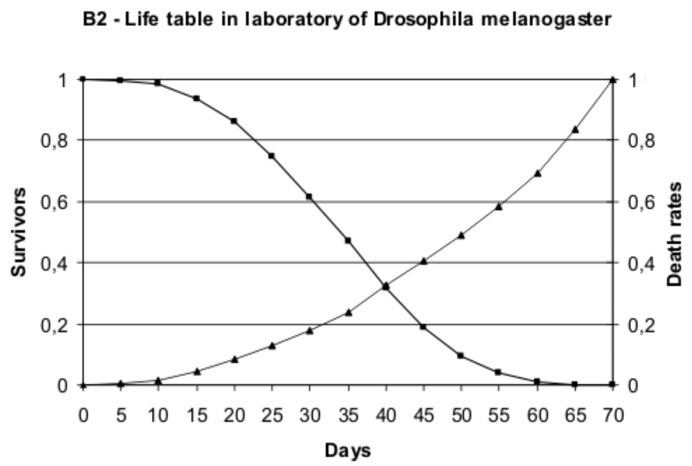


Figure 18. Life table and death rate of: (A1) lion (*Panthera leo*) in the wild, data from Ricklefs [9]; (A2) hippopotamus (*Hippopotamus amphibius*) in the wild, data from Ricklefs[9]; (B1) *Caenorhabditis elegans* reared in laboratory, data from Finch, Figure 6.1 [181]; (B2) *Drosophila melanogaster* reared in laboratory, data from Finch and Hayflick, Figure 10 [182].

A Surprising Cue: Animals with Negligible Senescence

"Negligible senescence" has been defined as the condition of species — such as rockfish, sturgeon, turtles, bivalves and possibly lobsters [189] — which show in the wild "no observable increase in age-specific mortality rate or decrease in reproduction rate after sexual maturity; and … no observable age-related decline in physiological capacity or disease resistance" [189]. In some cases fitness even increases with age, i.e. as a function of increasing body size [190].

For a theory explaining age-related fitness decline as the result of harmful factors that accumulate with the passing of time and are not sufficiently opposed by natural selection, the existence of animals reaching very old ages in the wild without any observable decline in their fitness, is a true challenge by no means solved by current theories of aging ("negligible senescence … may be in conflict with mathematical deductions from population genetics theory" [189]). For such a theory, ageless animals have to be explained as exceptions justified by hypothetical not documented physiologic peculiarities. Particular optimization models of life-history strategies, based on the suppositions of disposable soma theory [25,26] have been developed to justify even the cases of negative senescence [190].

On the contrary, for a theory explaining age-related fitness decline as caused in particular conditions by selective factors, there is a simple prediction: a species that is not in those particular conditions must be an ageless animal. This means that, for these species, survival in the wild (disregarding possible minor factors that modify fitness) is described by the simple formula:

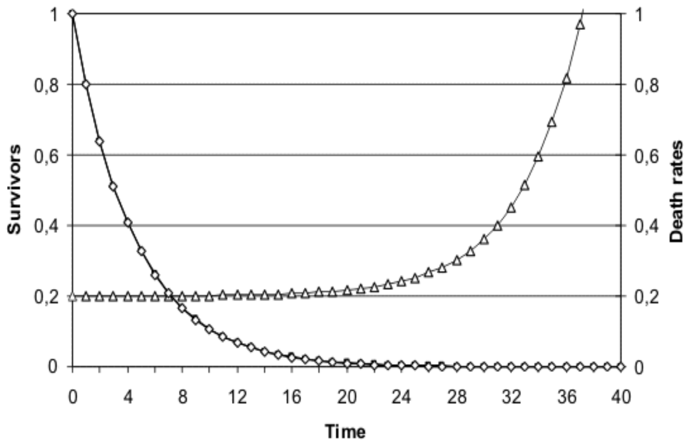
$$Y_t = Y_0 \cdot (1 - \lambda)^t \qquad (4)$$

where Y_0 is the initial population, Y_t are the survivors at time t and λ is the mortality rate.

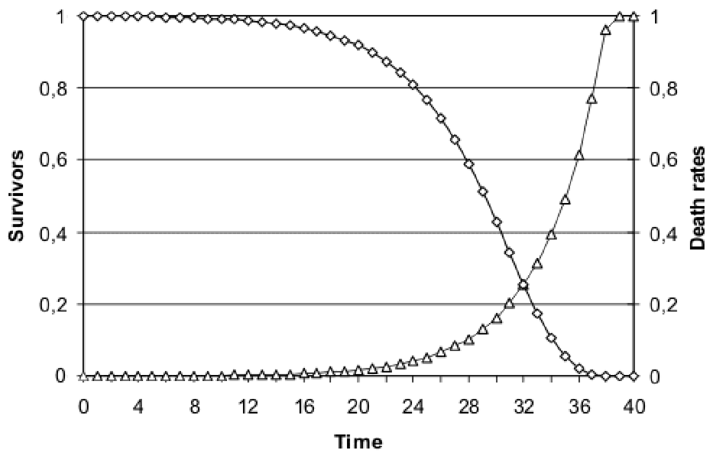
Survival is determined only by the parameter λ. With low values of λ, it is predicted that, in the wild, some individuals will reach very old ages. For example, if λ = 0,011306 / year, the survivors after 405 years will be about 1% and the case of *Arctica islandica* specimen with an age of 405 years retrieved near Iceland in 2007 will not result surprising.

However, at an age t reached in the wild by few or no individuals, there is very little or no natural selection against a gene with harmful action only at that age ("t-gene"). Therefore, by the cumulative effects of various "t-genes", if a species demonstrating no age-related fitness decline in the wild is reared in protected conditions, it could show a progressive increase in mortality starting from ages rarely or never existing in the wild. In other words, the species could show IMICAC phenomenon (Figure 19).

As a simple corollary of formula (4) and of this phenomenon, for a group of species of the same genus, all in conditions not favouring the fitness decline, it is predicted that: (1) all species will have a stable fitness at all ages existing in the wild, with a possible little decrement at ages rarely present in the wild; (2) for each species mean life span and maximum life span in the wild will be inversely correlated with λ; (3) in protected conditions, for each species life span will be determined by variable factors, which could increase mortality starting from ages rarely or never existing in the wild and therefore variable from species to species and inversely correlated with λ. Life tables of the rockfish genus are probably a good example of these predictions [191].



A



B

Figure 19. Results obtained with a simulation program (IMICAC.exe [40], available at the Internet address www.r-site.org/ageing/simprograms.zip). (A) A species with a constant extrinsic mortality rate in the wild (=0.2 / unity of time), plus the small increase of mortality rate, at ages rarely or never existing in the wild, due to the action of t-genes (20 in the simulation and with a mutation rate from inactive alleles equal to 0.00001); (B) The same species, in protected conditions with a zero extrinsic mortality rate, shows the IMICAC phenomenon due to the action of t-genes at ages not-existing in the wild.

It is interesting that for the rockfish genus, telomerase activity is constant at all ages existing in the wild [171,172]. Moreover, for two species of rockfish, it has been observed that oogenesis continues at advanced ages, in contrast with long-held assumptions [192].

A rockfish is showed in Figure 20.

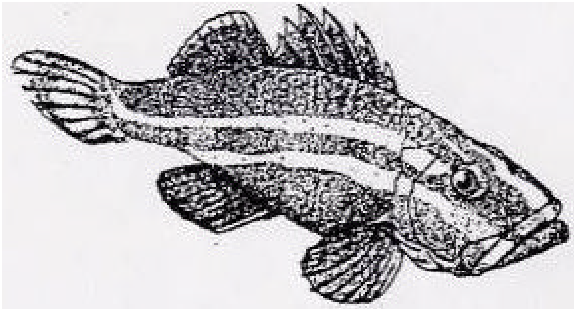


Figure 20. A rockfish. Image from the site www.agelessanimals.org, a scientific site, directed by John C. Guerin and dedicated to: Emerging Area of Aging Research: Long-lived Animals with "Negligible Senescence".

A Poorly Understood Cue: Aging as Distinct Entity

It is very common to hear from ordinary people that someone died due to old age. However, in the official statistics no one dies due to old age! In the compilation of official death certificates, in any nation of the world, a physician, as I am, must use the international classification of diseases (ICD), in which, though there are codes for “senile dementia” or “senile cataract”, a codification for “aging” or “senescence” is non-existent. Some years ago, I compiled the death certificate for a 102-year-old great-grandmother who had no particular disease, but I could not write that she was died because she was old!

A very authoritative gerontologist wrote to me on the subject, saying: “The question you are asking me is an old one - should senescence be listed on the death certificate of someone who dies past the age of 100.

For some people for whom it is not possible to find an underlying cause, such as individuals who experience what would appear to be a collapse of their entire body all at one, I would say that senescence would be an appropriate cause of death to place as the underlying cause.

I expect the frequency of this diagnosis will increase in the coming decades. However, should such a cause of death be added to the ICD, my guess is that it would overused by attending physicians too lazy to determine underlying cause.”

Aging as a distinct cause of death is disregarded or considered as non-existent by classic gerontological theories or by official epidemiology. For such theories, aging is not a specific process but only the sum of many different diseases (Figure 21). With this paradigm, we should cure each of these diseases, while the possibility of acting on aging is unthinkable because aging does not exist as a distinct entity!

Well, if you want to understand the next paragraphs, you should accept a new idea, a radical change of the old paradigm that “gradual decline in performance with age happens by default” [193]: i.e. aging as a distinct phenomenon exists!

Figure 21. According to the classic paradigm, these elders will not be killed by senescence but only by a myriad of diseases whose frequency increases with age.

Ancient and Current Scenery: Toward a Socio-Medical Nightmare

Until the beginning of the XIX century the child mortality rate was very high, the adult mortality rate was higher than present and the mortality rate for elders not very different from present. The following is an example of this historical child mortality rate. In the years 1812-1815, in the statistics from a part of the reign of Naples under king Murat [194] the number of those who died before 7 years of age was about 42-46% of the total of deaths, e.g.:

Table IV. Mortality in Naples and province at the beginning of XIX century

		< 7 years old	> 7 years old	Total
1812	(Province of Naples)	3.821 (42.62%)	5.144	8.965
1813	"	4.420 (45.44%)	5.308	9.728
1814	"	4.367 (45.24%)	5.287	9.654
1815	(City of Naples)	5.600 (42.22%)	7.664	13.264

In the next 200 years with the large improvement in economic and hygienic conditions and with the advances in medical cure, there has been a drastic lowering of children's mortality, a strong reduction of adult mortality and a relatively modest increase in life expectancy for the elders. The triplication of mean life span in these two centuries (from about 25 years in 1800 to about 75 years today) is due largely to a drastic reduction in children's mortality and contrasts strongly with an apparently stable maximum life span. This is shown for England in Figure 22, which indicates that the survival table is becoming similar to that of a straight line followed by a sharp drop (rectangular curve). With the increasing control of tumoral, cardiovascular and other diseases (and an exponential increase in related costs), a greater rectangularization of the survival curve is a realistic forecast for the next decades.

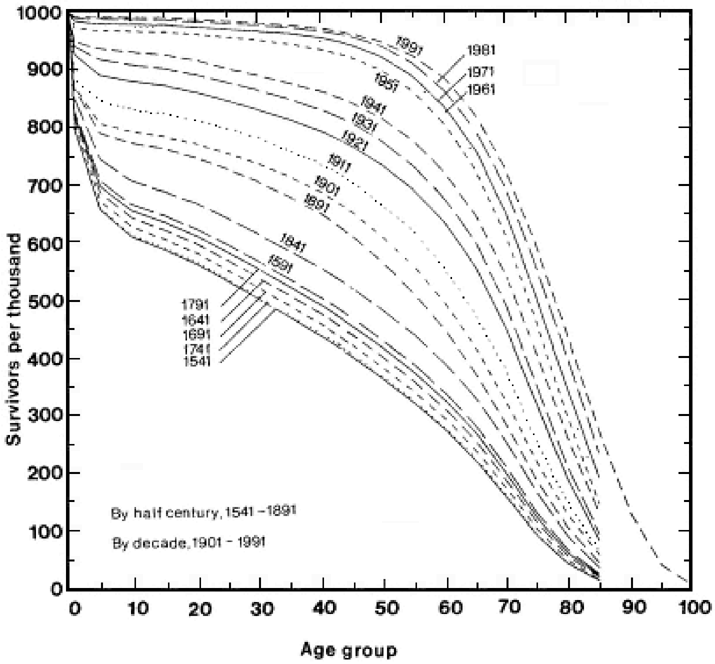


Figure 22. Survival curves for cohorts of one thousand newborns, by age group: England, 1541-1991. Data from Cambridge Group back projection files and English Life tables up to no. 15. Work of James Oeppen. From: Kertzer, David I., and Peter Laslett, editors *Aging in the Past: Demography, Society, and Old Age.* Berkeley: University of California Press, c1995 1995. http://ark.cdlib.org/ark:/13030/ft096n99tf/.

This means that the population will be comprised of an increasing number of elderly individuals with severe troubles and pains deriving from marked osteoporosis, harsh cardiovascular and respiratory insufficiency, senile dementia, visual and auditive deficits, incontinence, etc. In short, an increasing part of the population will be seriously suffering from many diseases, in particular for the decay of cognitive and sensory capacities, and they will therefore be dependent on others. This will have heavy economic results. The progress of medicine will become the cause of a sociological and economic nightmare.

In a sense it will be the realisation of the legend of Aurora and Tithonus. Aurora, a goddess, obtained the gift of immortality from Jupiter for her beloved Tithonus, a mortal man, but neglected to ask for perpetual youth. Tithonus became older and older and never died. Lastly, out of pity, Tithonus was transformed in an animal. In 1979, Comfort said: “We are producing Tithonuses” [195]!

Today, we could say that a mass production of Tithonuses has been started.

A Possible Alternative Scenery: Toward the Taming of Senescence

We have two opposite general hypotheses, or paradigms, about the fitness decline and its extreme expression, i.e. 'old age' or the 'senile state'.

For the first paradigm, the phenomenon is something inescapable, inevitably inherent to the nature of life and weakly opposed by repair mechanisms in the tight limits of other prevailing demands dictated by natural selection. To counter senescence is as to oppose the force of gravity in the construction of a skyscraper: the higher one goes, the exponentially greater are the necessary efforts. In other words, the undertaking of this task becomes impossible beyond certain heights.

For the second paradigm, fitness decline is a function: suitable mechanisms, which are genetically determined and favoured by evolution because of their positive inclusive fitness, limit life span. Opposing senescence is like removing the obstacles or limiting the friction beneath a ball that rushes on a flat surface. Clearly the natural condition of such a moving body - in absence of friction and other obstacles - is an unlimited movement.

According to the first paradigm, the interpretation of senescence as a program is an absurdity because it would be of no evolutionary meaning. Therefore, the growing evidence in support of such a program is disregarded and attention is fixed only on the stochastic accumulation of damages of various types.

Conversely, according to the second paradigm a program is not at all excluded. In fact, without a program the second paradigm would be false, and attention is fixed on the events that actively determine and regulate the progressive fitness reduction. Moreover, as aging is the consequence of genetically determined mechanisms and not the sum of stochastic events, such mechanisms are the rational object of useful analysis and of possible control and modification. What for the first paradigm is an insuperable obstacle and a closed horizon, for the second paradigm is a modifiable and controllable trait, one with a limitless horizon.

To master the senile state, apart from foreseeable crucial objections of bioethical or philosophical or religious nature that will be outlined immediately after, three categories of action are required:

1) *Alterations due to cell turnover limitations* - A thorough knowledge of the mechanisms underlying cell turnover and its limitations are needed. Currently, drugs with "organ protection" properties seem to act efficaciously on some cell turnover alterations [117]. It seems more rational to propose as a future treatment the modification of regulating genes before the onset of aging manifestations, in a sort of gene "therapy". Incidentally, the term "therapy" is open to criticism since age-related fitness decline not properly a disease. Ten years ago extraordinary experiments demonstrated that the insertion of an active telomerase gene or, in general, telomerase activation, eliminates replicative senescence and the effects of cell senescence [65,66,69]. This indicates that the effects of many factors on aging, oxidative substances included, are reversible consequences of cell senescence and not the cause of aging [74].

Presently, gene therapy is possible, or in trial, for only a few diseases and with the insertion of the appropriate gene in a random DNA position [196,197,198], therefore with the possible noxious modifications of other genes, e.g., oncogene suppressors (Figure 23). This is a strong contraindication for an indiscriminate use of this therapeutic technique.

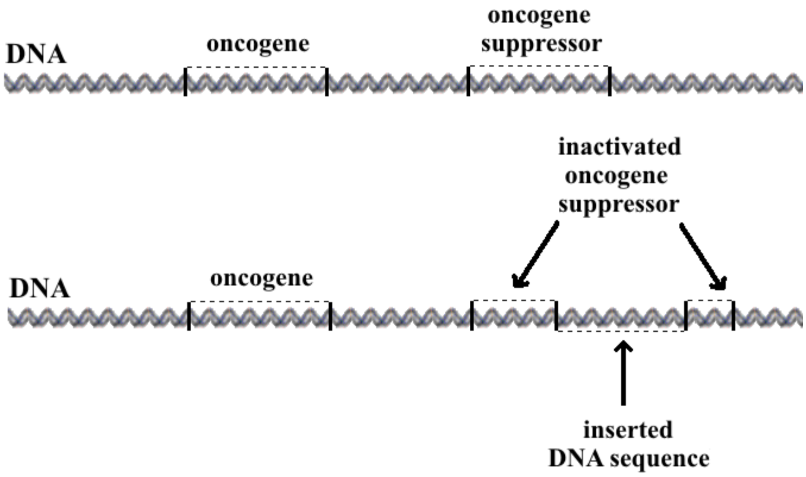


Figure 23. The insertion of a DNA sequence in a random point may cause damage that is fatal for the whole organism, e.g. inactivating an oncogene repressor.

Furthermore, to limit this danger, the gene is inserted in only a fraction of cells that, if somatic, are substituted by turnover, which gradually cancels the therapeutic effects. Ideally, gene therapy should recognise a known sequence with no function and present as unique copy in the whole DNA and then, with a safe vector, insert the gene, breaking the known sequence in a precise point so that a second insertion would be impossible because the known sequence is modified (Figure 24). Moreover, the gene should be inserted in the majority, or prefereably all, cells (stem cells included) so that its elimination by cell turnover would be avoided. With these specifications, apart from any possible bioethical objections, gene therapy in non-germ line cells could be proposed to modify telomere-telomerase system so that age-related fitness decline will be postponed or even nullified.

Later, the possible application of the same techniques to germ line cells, namely the possibility to obtain a status of “negligible senescence”, probably will become only a bioethical / philosophical / religious problem.

2) *Age-associated diseases* – At ages rarely or never present in the wild, natural selection against genes causing a disease is weak or non-existent. Such diseases can be defined as “age associated disease” because they are an evolutionary consequence of age-related increasing mortality in the wild. A thorough knowledge of each of the associated diseases is a plain preliminary condition. The next step is the achievement of treatments to completely control each of them, namely avoiding that with growing age their harm, even if reduced, accumulates. However, as age-associated diseases are very common in the elderly [199] and the coexistence of diverse age-associated diseases in the same individual is common [199]. For this reason, the modification of the altered genes before the beginning of the symptoms seems the ideal treatment. About gene therapy, see the preceding paragraph.

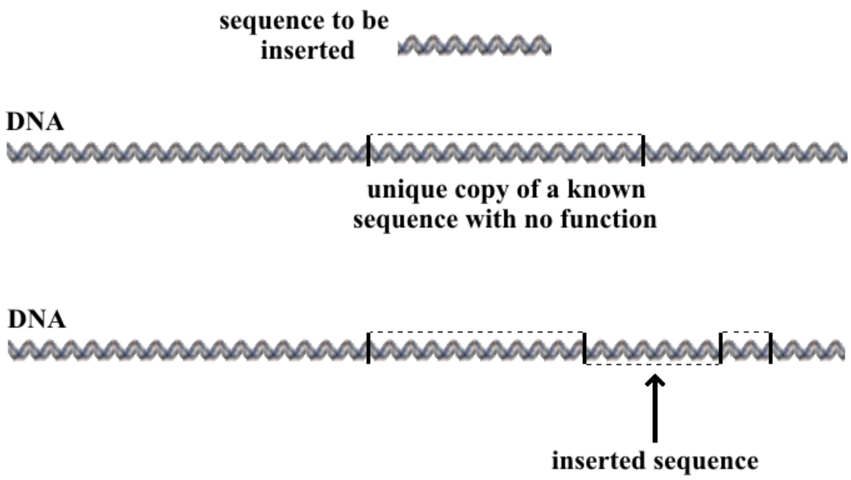


Figure 24. The sequence is inserted breaking a known sequence existing as a unique copy in the whole genome and with no function. The breaking of this unique sequence makes impossible another gene insertion in the same cell.

3) *Alterations due to wear and tear factors* - The main example of this is tooth wear. Besides their replacement with prosthesis, multiple dentitions, namely a periodic tooth renewal as is found in other species, is imaginable. "The senescence of human teeth consists not of their wearing out but of their lack of replacement when worn out." [23]. Thus period tooth renewal would be possible by means of germ line cell modifications, although the aforementioned ethical objections would still remain.

Bioethical / philosophical / religious objections. The possible treatments mentioned above pose great technical obstacles, none of them in principle insuperable, but there are two much greater problems, the solutions to which are not at all scientific or technical (Figure 25):

I. The first is that to modify natural aging to a slower or even a zero rate (negligible senescence) constitutes an enormous change of human nature, and is not merely the correction of a disease. For this, and even more for any hypothesis of germ cell modification, it is easy to anticipate strong bioethical, philosophical or religious objections, or even accusations of blasphemy[Note 1] or of ὑβρις[Note 2].
II. The second, still greater, difficulty, is that changes in civilisation resulting from senescence slowing or even from a non-senescent condition, would certainly be extreme and full of uncertainties. The roots of our civilisation, organisational structure and cultural traditions are based upon the philosophical idea and religious creed that life span limitation is ineluctable. The drastic change of such a reality would be a revolution greater than any other revolution ever experienced by our species.

Figure 25. To act, or not to act on telomere-telomerase system: that is the question.

The Need of a New Dramaturgy: Conclusion

The change of an ancient paradigm seems indispensable. Age-related increasing mortality is not an unavoidable fate, as the old paradigm claims. To the contrary, it is a trait, the underlying mechanisms of which can now be described in precise detail, which has been actively moulded by selection. Although such a trait seems paradoxical because it does not improve individual fitness, the hypothesis that it has been moulded by selection means that it is modifiable and tameable in principle. The old paradigm considers fitness decline to be a maladaptive character that can only be partially opposed [200]. By considering aging as “a specific biological function” [42], then, the new paradigm offers a more optimistic approach to treating aging.

However, the change of a paradigm is always a scientific revolution and this usually requires a new generation [201].

Note 1) "Then God said, 'Let Us make man in Our image, after Our likeness ...." Genesis 1:26; "So God created man in His own image, in the image of God He created Him ..." Genesis 1:27.

Note 2) In the Greek classic culture a mortal that presumed to measure himself against the gods, regarding himself or searching for being like to them, became guilty of ύβρις, namely of unforgivable impious pride and arrogance toward the deity.

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Reviewed by:

Prof. Vladimir P. Skulachev,
Sc.D., Head of Department,
Academician of Russian Academy of Sciences