Medicine Papers, 2016, 2 (2): 29–44

Bio-genetic theories of ageing

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Summary

During the twentieth century, understanding of the significance and function of nucleic acids, DNA and RNA, and core mechanisms of the cell as well as discovery of antibiotics and, consequently, increasing progress of pharmacology, have determined, especially in industrialized countries, a radical change in the conditions and duration of life. Currently, in Italy, the average life expectancy is 80.1 years for men and 84.7 years for women. Although these data (unthinkable 100 years ago) represent an extraordinary outcome, nevertheless, they also pose new social, health and economic challenges. In fact, since the improvement in life expectancy is necessarily associated with a significant increase in the incidence and prevalence of diseases typical of the elderly, the study of biological and physiological features of ageing may hopefully provide useful tools to give relief to the pathological and, eventually, painful aspects of old age. In this context, our review addresses the main theories of ageing: genetic, unbalanced homeostasis and cell damage theories. However, it should be stressed that these theories (in spite of their monistic attitude) do not exclude each other and that ageing and longevity are to be considered multifactorial and very likely modulated by a combination of endogenous and exogenous factors, even if with some elements bearing more importance than others. Moreover, although organisms resemble in many ways, living systems are very heterogeneous and the maximum lifespan could even be determined by completely different factors in diverse species.

Key Words

Ageing; Lifespan; Genetic theories; Neuroendocrine theory; Immunological theory; Free radical/Oxidative stress theory; Caloric restriction; Glication theory; Membrane theory; Prions.

Received 08.04.2016; accepted 13.05.2016; printed 30.05.2016

Introduction

Ageing is a natural and progressive process: biological and morphological changes occur in vital organs such as the brain, lungs, heart and blood vessels (Beers & Berkov, 2000; Fillit et al., 2010). At the same time, psychological ageing is characterized by declining cognitive efficiency - especially for the short-term memory and selective attention - relational and affective space shrinkage, increased sensitivity and difficulty in adapting to new situations.

The chronological beginning of ageing, very variable, is also influenced by socio-cultural factors and its progression by both the elder personality and environment.

In recent years, ageing has become the focus of the interests and efforts of researchers worldwide, main reason being the growing ageing population of the industrialized countries. According to the World Health Organization in 25 years' time more than 65 years old people will reach 10% of the world's population. In Italy, the population of the "over 65" is already at 17%, with the prospect of arriving at 23% in 2020. While at the beginning of the 20th century Italian life expectancy was 43 years for women and 42.6 for men (without any relevant variation with respect to past centuries), today the average life expectancy is 80.1 years for men and 84.7 years for women (data from www.istat.it). Hence, new and important issues are to be undertaken by health and social services, in order to deliver appropriate treatment and care for age-related chronic diseases to elder people.

Although taking into account individual variables, ageing could be considered as a progressive decline of various systems:

Cardiovascular system - Shape, volume and weight of the heart change. Between 30 and 90 years of age, the weight increases at a rate of 1 - 1.5 grams per year; the fibers are decreasing in number and size, the valves become stiff, aortic or mitral calcifications often develop; ventricular walls thicken. Coronary and all arterial and venous vessels undergo alterations inducing walls rigidity. Dilation of post-capillary venules decreases venous tone and reduces blood flow from periphery to the heart. In the venous system, augmentation of connective tissue and corresponding diminution of elastic fibers promote blood stasis, especially in lower limbs. Both diastolic and systolic blood pressure increase. The aerobic ability to burn oxygen under stress decreases by 10% every 10 years in men and 7.5% in women.

Respiratory system - Over the years, the pulmonary tract undergoes a series of changes that lead to the formation of the so-called "senile lung": ribcage alterations, bronchial mucosal atrophy, dilation of pulmonary alveoli, blood vessel constriction and sclerosis. Vital capacity (the maximum volume of inspired air) decreases by 40% from 10 to 70 years of age.

Kidneys and urinary tract - Kidneys gradually become less efficient at blood filtering. Moreover, the bladder reduces its volume and atrophy of the tissues can lead to urinary incontinence.

Muscular tissue - In the absence of constant exercise, from 30 years of age muscular fibers decrease by 3 - 5% every decade; muscular tone and elasticity declines; stiffness of muscles can cause limitations in movement range and in physical work ability.

Osteoarticular system - Synovial fluid becomes less viscous, tendons stiffen, capsules and ligaments lose their elasticity, thus reducing the range of joints movement. As far as concern the bone system, the altered dynamic balance between renewal and deconstruction processes results in bone mass decreasing (osteopenia) with ageing. These phenomena occur slowly and regularly in men, suddenly in postmenopausal women. In severe cases, the total reduction in bone density may cause osteoporosis.

Central nervous system - Over time, the number of efficient neurons drops, although the phenomenon is partially balanced by both increasing of connections (synapses) between cells and development of neuron axons and dendrites.

Visual system - Muscle atrophy of the crystalline lens makes it more difficult to focus close objects (presbyopia). Also, to distinguish objects in movement or in reduced light conditions becomes more difficult from 50 years of age.

Hearing system - The ability to perceive higher frequency sounds declines.

Cutaneous system - Sweat glands decline, skin sensitivity decreases, skin wrinkles appear because of loss of elasticity.

All of these macroscopic effects of ageing are the result of genetic, biochemical and physiological processes.

Why do we age?

In the late 19th century, August Weismann theorized that the ageing of bodies and their cells originated from the same cellular differentiation processes by which the entire organism is built. Alexis Carrel, at the beginning of 1900, considered ageing an attribute of the whole multicellular body rather than a property of the single cell.

One of the first theories on the phenomenon of ageing, tried to link the maximum extent of life of various mammals with four anatomic and functional variables: i) weight of the adult brain, ii) body weight, iii) metabolism rate and iv) body temperature. A complex equation was formulated, by which longevity seemed directly related to the index of cephalization, expressed as brain-body ratio (the higher the ratio, the higher life expectancy), and inversely correlated with metabolism rate (the faster metabolism, the more rapid ageing and death) (Hansche, 1975).

Instead, the entropy theory proposed that higher organisms, considered organized and complex systems, were intrinsically unstable and predisposed to deconstruction: thus, ageing would be the fate of a complex system as the human being.

Biological theories of ageing can be divided into three main groups (Hayflick, 1985; Lipsky & King, 2015; Sergiev et al., 2015): genetic, unbalanced homeostasis and cell damage theories.

Genetic theories suppose that ageing, as well as any other phase of life (i.e., puberty and menopause), could be genetically programmed or, however, caused by accumulation of changes in the genetic pool. On the contrary, cell damage theories focus on the influence that the environment exerts on the organism. Finally, unbalanced homeostasis theories argue that ageing is a consequence of the gradual loss of efficiency of the neuroendocrine and immune systems.

Genetic theories

As well as external factors may affect the ageing process, also the internal ones can play a role in the progression of senescence. Most of scholars seem to agree in saying that ageing is the result of genetic and environmental components, even if their relative importance can be differently weighted. Thus, gene researchers suppose that most of the causes responsible for ageing should be searched for in genome variability and regulation.

Studies conducted on yeast, on the nematode *Caenorhabditis elegans* (a common soil worm) and on the Diptera *Drosophila melanogaster* (the fruit fly), have shown that several genes could be linked to ageing or longevity. Nevertheless, identifying a particular gene is only a first step that should be followed by understanding how it exerts its biological activity and what could trigger it, particularly in complex system like mammals and humans.

The Hayflick theory was based on the interpretation of experiments carried on *in vitro* culture of fibroblasts, and in many other tissues as well (Hayflick & Moorhead, 1961). Hayflick found that *in vitro* cultured human fibroblasts could have roughly 50 cycles of replication before entering a senescence phase, which, after about 10 more divisions, led to extinction of the colony. He was also able to show that, in various animal species, number of fibroblast replications was proportional to their species expected lifespan, thus suggesting that life expectancy could be linked to genetic factors, and that each species and, in particular, each individual of the species had a programmed "inner clock". Since Hayflick studies organism ageing is supposed to be the result of cells senescence, although it's not been properly characterized which mechanisms would link cellular and organism senescence.

Some scientists argue that cultured cells cannot mimic ageing of the organism. According to this hypothesis, *in vitro* ageing promoting factors could most likely be very dissimilar from those affecting *in vivo* senescence (Holliday, 1990; Johnson, 1997; Rubin, 2002). Nevertheless, cell cultures have proved to be a very reliable and useful tool in (i) studies of cell terminal differentiation, (ii) defining aspects of cell cycle control and (iii) analysis of oncogenesis mechanisms.

Another theory assume that, since cells can only divide a certain number of times before dying, apoptosis (programmed cell death) would be an important cause of ageing. The cells of a multicellular organism are members of a highly organized community. The number of cells in this community is tightly regulated, not simply by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they commit suicide by activating an intracellular death program. The process of programmed cell death is generally characterized by distinct morphological features and energy-dependent biochemical mechanisms (Elmore, 2007). Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Inappropriate apoptosis (either too little or too much) is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. Evidence suggests that mutations that give rise to features resembling premature ageing tend to be associated with cellular phenotypes such as increased apoptosis or premature replicative senescence. In contrast, many interventions in mice that extend lifespan and might delay ageing tend to either hinder apoptosis or result in smaller animals and thus may be the product of fewer cell divisions (De Magalhães & Faragher, 2008). To verify this hypothesis, some scientists focused their studies on regulation of p66 gene. This gene confers increased resistance to oxidative stress and encode an adaptive protein involved in cell signaling. In murine models, induced p66 deletions were found to be directly related to a decrease in apoptosis rate resulting in a 30% lifespan increase of tested mice (Migliaccio et al., 1999; Purdo & Chen, 2003). Therefore, it appears plausible that changes in the number of times that cells, and particularly stem cells, divide during an organism's lifespan influence longevity and ageing.

Genetic theories are often also referred to as Programmed Theories of Ageing because they hypothesize that some regulatory genes, activated according to a predetermined project, would cause the changes usually observed during senescence. Several data would confirm such genetic control of lifespan: 1) lifespan diverges greatly in different animal species; 2) existence of long-lived families in our species; 3) human genetic diseases (i.e. Progeria, Down syndrome) characterized by an acceleration of the ageing process and a reduced lifespan.

In such context, *Caenorhabditis elegans* was chosen as an optimal experimental model because of its limited number of genes, easily identifiable. In presence of the mutated gene age-1, the worm life is 65-70% longer, while the resistance against oxidative stress, thermal stress and ultraviolet radiation increases (Johnson, 1990). The age-1 mutant strains

move better at all stages and in old age, suggesting that such mutation also increases the worm longevity, vitality and health (Duhon & Johnson, 1995). Age-1 maps to chromosome 2, halfway into a genetic region of 150 kb and appears to have little effect on fertility, on length of reproduction and on development rate (Friedman & Johnson, 1988; Johnson & Lithgow, 1992; Johnson et al., 1993). These data seem very likely to suggest the existence of "longevity assurance genes" rather than “gerontogenes” (genes affecting ageing) (Rattan, 2004).Studies on centenarians have shown that long-lived people have higher prevalence of certain genes than common population. Moreover, this long-living feature would be more likely determined by a complex genetic pattern rather than by few isolated genes. Particularly, among human centenarians a higher frequency of mtDNA haplo-Group J and an increased allelic occurrence of genes HLA DR11, HLA DRB1 and HLA DQ were found. Researchers of the Boston Children's Hospital showed that families of long-lived persons have a series of specific genes on chromosome 4 (Puca et al., 2001).

Other studies as well highlighted the important role of hereditary component in long-living feature (Perls et al., 2002; Barzilai et al., 2003; Couzin, 2003; Perls & Terry, 2003; Weverling-Rijnsburger et al., 2003). Moreover, several “biological” markers were described in centenarians: i) frequency of the O blood type (associated with a reduced incidence of cardiovascular disease); ii) high concentrations of HDL (high density lipoprotein) cholesterol (responsible for removing cholesterol from vessels); iii) large amount of lipoprotein(a) (associated with prevention of stroke and heart attack); iv) low blood pressure levels (blood vessels elasticity protection).

Researchers also focused on studying, by means of DNA microarrays, genes functioning in youth, in order to avoid their inactivation and, thus, to prevent or, at least, slow down the ageing process (van Gansen & van Lerberghe, 1988; McGrath, 2002).

Telomeres are fragments of DNA located at the ends of chromosomes whose length is reduced with age. Telomeres and Telomerase (a ribonucleoprotein enzyme complex necessary to preserve the Telomere integrity) are essential to cellular proliferation regulation. The telomere, located at both ends of the chromosome, structurally is a pool of short identical DNA sequences repeated thousands of times in tandem (consecutive). These repetitive terminal sequences are devoid of genes and have therefore not coding properties. The telomere function has not yet been definitively established, although it is likely to be crucial for proper functioning of all other genes. In most eukaryotes, including humans, the telomeric sequence is (TTAGGG)n, where n represents the number of repetitions (on average about 2000 in humans).

Telomeres shorten whenever chromosomes are duplicated during the S phase of cell division (mitosis). The telomerase enzyme, if present, can restore lost repetitive sequences, returning the telomere to original dimensions. However, in normal conditions, telomerase is present and active only in the germ cells (sperm, egg cells and their progenitor cells) and during embryonic development, thus ensuring full telomeres occurrence at birth. In later life, telomerase disappears in nearly all somatic cells, therefore occurring a shortening of telomeres which is related to the number of cell replications (mitotic clock). In the last phase, telomeric regression associates with the phenomenon of cellular ageing (replicative senescence).

When a critical threshold of telomeres shortening is reached after a given number of duplication (50-70 in human cells), cell growth is blocked and, because of the fragmentation and fusion of chromosomes (chromosome instability), from a stage of senescence cells enter a lethal phase.

It is estimated that 50-70 cells generations are enough to keep a person healthy for about 100 years (unless other disturbing events happen). According to some scholars, the replicative senescence could be the condition to explain both whole organism ageing and age-related diseases onset.

Although the link between telomeres shortening and senescence has long been described (Bodnar et al., 1998; Sharma et al., 2003), this mechanism is not yet clear. Recently, it was found out that when telomeres are too short, a specific emergency system, triggered by DNA damage, block cell replication (D'Adda Di Fagagna et al., 2003; Campisi & D'Adda Di Fagagna, 2007). The study of this system could help assessing if senescent cells are linked to ageing (for example, by studying whether senescent cells accumulate in the elderly) and how genomic instability is involved in cancer onset.

Genes involved in genome stabilization could be considered strong longevity assurance genes candidates (Sacher, 1982). This is dramatically showed by the segmental progeroid syndromes, which are characterized by an accelerated emergence of several aspects of the senescent phenotype and by the premature onset of several age-associated pathologies: Ataxia Telangiectasia, Werner syndrome, Cockayne syndrome, Bloom syndrome, Hutchinson-Gilford syndrome and Fanconi anemia (Vidak & Foisner, 2016). Notably, most of these syndromes

are caused by inherited mutations in genes involved in DNA processing and repair.

The protein deficient in Werner syndrome is a RecQ-type DNA helicase involved in DNA repair, replication, telomere maintenance and transcription (Cheng et al., 2007). Other diseases caused by inherited mutations in a helicase are Bloom and Cockayne syndromes. Bloom syndrome is a rare autosomal recessive genetic disorder characterized by growth deficiency, unusual facies, sun-sensitive telangiectatic erythema, immunodeficiency and predisposition to cancer (Kaneko & Kondo, 2004). Although Bloom syndrome shares some important features with Werner syndrome (such as high level of somatic mutations in the patients white blood cells and high rate of somatic recombination), the latter presents with much more signs and symptoms of accelerated ageing. Indeed, while Bloom syndrome is primarily associated with an increased risk of cancer, Werner syndrome patients have greater risk for arteriosclerosis, cancer, osteoporosis and diabetes type 2, in addition to early hair loss and premature graying, skin atrophy and cataracts.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by a dramatic appearance of premature ageing (from 5 to 10 times faster than normal) causing death before the age of 20 years due to cardiovascular problems and heart failure (Vidak & Foisner, 2016). HGPS is linked to mutations in the LMNA gene encoding the intermediate filament protein lamin A (De Sandre-Giovannoli et al., 2003). Lamin A is a major component of the nuclear lamina, a scaffold structure at the nuclear envelope that defines mechanochemical properties of the nucleus and is involved in chromatin organization and epigenetic regulation. Lamin A is also present in the nuclear interior where it fulfills lamina-independent functions in cell signaling and gene regulation. The most common LMNA mutation linked to HGPS leads to mis-splicing of the LMNA mRNA and produces a mutant lamin A protein called progerin that tightly associates with the inner nuclear membrane and affects the dynamic properties of lamins. Progerin expression impairs many important cellular processes providing insight into potential disease mechanisms. These include changes in mechanosignaling, altered chromatin organization and impaired genome stability, and changes in signaling pathways, leading to impaired regulation of adult stem cells, defective extracellular matrix production and premature cell senescence.

Finally, two other genetic theories deserve attention. According to the Theory of Accumulation, ageing is a consequence of the accumulation of mutations in some genes, controlled by reproductive selection processes during the early stages of life, but later activating and determining a very serious impairment of cell functions (Ferraro & Morton, 2016).

The Theory of Antagonistic Pleiotropy argues that ageing would be determined by the action of genes earlier playing a crucial role in reproductive age (such as those that produce estrogen), but later assuming a deleterious role (Williams, 1957; Turke, 2008). Researchers of the University of Illinois used mathematical models based on these two theories to estimate reproductive success at different ages in 100 genotypes of *Drosophila melanogaster* (Hughes e al., 2002; Hughes & Reynolds, 2005). Obtained data showed that accumulation mechanism was the most significant although a possible contribution by the antagonistic pleiotropy mechanism could not be ruled out: mutations deleterious effects on reproduction would have dramatically increased with age during the reproductive years. Hence, hypothetically, it was pointed out that the ageing process might have been slowed down by modulation and/or inactivation of mutated genes that are switched on in old age.

On the whole, genetic association studies suggested that, also in humans, mutations in genes correlated with the maintenance of the cell and of its basic metabolism are essential in modulating lifespan. Indeed, genes involved in DNA repair (Debrabant et al., 2014), telomere conservation (Soerensen et al., 2012), heat shock response (Ross et al., 2003), and the management of free radicals' levels (Raule et al., 2014) were found to contribute to longevity or, in case of reduced functionality, to accelerated senescence (cellular ageing) and the consequent organism ageing. In addition, as suggested by the studies in mice, the pathways involved in nutrient-sensing signaling and in regulating transcription, such as IGF-1/insulin axis (Junnila et al., 2013) and TOR (target of rapamycin) (Johnson et al., 2013) showed to be involved in modulating human longevity.

Besides, also genes involved in lipoprotein metabolism (especially APOE), cardiovascular homeostasis, immunity, and inflammation have been found to play an important role in ageing, age-related disorders, and organism longevity (Passarino et al., 2016).

Epigenetic changes involve transmissible alterations in gene expression caused by mechanisms other than changes in DNA sequence. Such variation is transmitted by cell division, but generally not passed on through the germ line. While, in the past, human biology was considered the interplay between

the DNA sequence of one's genome and environmental variation, evidence has been accumulating that epigenetic modifications as DNA methylation and chromatin remodeling driven by histone modifications can have a primary role in phenotypic outcomes, including human disease and ageing (Gravina & Vijg, 2010). Indeed, epigenetics has been considered to be at the "*epicenter of modern medicine*" (Feinberg, 2008) because it explains the relationship between an individual's genetic background, the environment, ageing, and disease. While epigenetic changes are essential for development and differentiation, they can also arise later in life either by non-random mechanisms, such as responses to environmental change, or through stochastic errors in maintaining fixed patterns of DNA or histone modification. Despite the fact that the Epigenome (the multitude of chemical compounds and proteins that can attach to DNA and direct such actions as turning genes on or off, controlling the production of proteins in particular cells) is highly dynamic, it is also strictly controlled. The loss of this control would be detrimental and its relaxation can be one of the causes of age-related diseases. Thus, ageing could be, at least in part, considered a time-dependent, epigenetically mediated loss of phenotypic plasticity. However, many questions regarding epigenetics and its role in ageing and age-related diseases still remain open. Do epimutations accumulate stochastically with ageing in different tissue types and what are, eventually, the consequences of this accumulation? Which genes are responsible for enhanced disease susceptibility when epigenetically deregulated, which environmental factors are deleterious for the epigenome and, finally, which epigenetic biomarkers could be used for detection of early-stage diseases?

In conclusion, it should be stressed that almost all assumptions proposed so far imply directly or indirectly the existence of a universal monistic cause of ageing common to all living species. Nonetheless, according to some authors, although organisms resemble in many ways, living systems are very heterogeneous: therefore, the maximum extension of life could be even determined by completely different factors in diverse species (Semsei, 2000).

Theories of unbalanced homeostasis

Human ageing is a slow process, not always quite assessable by chronological age; moreover, it is very difficult to choose appropriate and reliable criteria to study it. Among individuals there are marked differences in the decline of physiological functions and, even in the same person, different functions may be differently affected during time. Hence, scholars applying the unbalanced homeostasis model to study ageing proposed neuroendocrine and immune systems as much more reliable indicators.

The Neuroendocrine Theory. Neuroendocrine theory focuses on the idea that a sort of biological clock controls production and activity of hormones: in this context, ageing would be the result of their decline. This theory considers the senescence to be an expression of progressive functional unbalance of neuroendocrine system. This system consists of a nervous component (the hypothalamus) and of several endocrine glands and organs: the pituitary gland (connected to the hypothalamus), the gonads, the thyroid and parathyroid glands, the adrenal glands and the pancreas. The neuroendocrine system works as a closed circuit, with mutual interference and control mechanisms modulating hormones production to cope with organism needs and varying by day-time and different circumstances (hot/cold, food/fasting, activity/resting, sleep/wake, stress conditions). A such subtle biological machinery can over time undergo functional alterations that may affect organs and systems. Indeed, many observations document an alteration of the hormonal production with advancing age (Gupta & Morley, 2014), as well documented for the severe declining of female sex hormones that occurs after menopause. Testosterone, the main male sex hormone, undergoes an apparently similar trend (decreasing of blood levels) with age (andropause or male menopause), but this change is quite variable among individuals and do not seem to fit the pattern described for menopausal women. Nevertheless, several studies showed that testosterone low blood levels could be significantly related to an increasing risk for coronary heart disease, while higher levels of this hormone would be associated to more availability of blood HDL cholesterol. Thus, testosterone deficiency might facilitate arteriosclerosis in elder men (English et al., 2000; Dobrzycki et al., 2003). Moreover, testosterone administration to middle-aged men would be associated with decreased visceral fat and glucose concentrations and increased insulin sensitivity (Bhasin, 2003).

Finally, in a recent study on 790 men 65 years of age or older, raising testosterone concentrations for 1 year from moderately low to the mid-normal range for men 19 to 40 years of age, had a moderate benefit with respect to sexual function and some benefit with respect to mood and depressive symptoms but no benefit with respect to vitality or walking distance.

Notably, the number of participants was too few to draw conclusions about the risks of testosterone treatment (Snyder et al., 2016).

Dehydroepiandrosterone (DHEA), an adrenal androgen, has also attracted much attention as an anti-ageing hormone as well as a marker for senescence because of its unique change along with ageing. DHEA is reported to have beneficial effects such as anti-diabetes, anti-obesity, and anti-atherosclerosis (Perrini et al., 2005). Moreover, declining blood levels of DHEA were related in man to a higher risk of cardiovascular disease (Savineau et al., 2013). Nonetheless, the use of DHEA for preventive or therapeutic purposes in the general population is not yet allowed outside controlled clinical studies. Indeed, this treatment could have unpleasant and/or dangerous side effects like hirsutism and increasing of ovarian cancer risk in woman and increasing of prostate cancer risk in man.

Growth hormone (GH) and melatonin also seem to prevent cardiac ageing, as they contribute to the recovery of several physiological parameters affected by age. These hormones exhibit antioxidant properties and decrease oxidative stress and apoptosis (Paredes et al., 2014).

In a famous study, 12 healthy men from 61 to 81 years old, receiving a six months GH treatment, showed an 8.8 percent increase in lean body mass, a 14.4 percent decrease in adipose-tissue mass, a 1.6 percent increase in average lumbar vertebral bone density and a 7.1 percent increase in skin thickness (Rudman et al., 1990), leading the scientists to conclude that diminished secretion of growth hormone could be responsible in part for the decrease of lean body mass, the expansion of adipose-tissue mass, and the thinning of the skin that occur in old age. However, to date, clinical studies have not demonstrated a clear clinical benefit of growth hormone replacement in normal ageing subjects (Thorner & Nass, 2007). Moreover, GH continued use could result in numerous side effects: carpal tunnel syndrome, high blood pressure, diabetes. Therefore, currently, the use of recombinant human growth hormone is only advised for children's growth deficit.

Melatonin, the neurohormone of the pineal gland, associates with molecules and signaling pathways that sense and influence energy metabolism, autophagy, and circadian rhythms, including insulin-like growth factor 1 (IGF-1), Forkhead box O (FoxOs), sirtuins and mammalian target of rapamycin (mTOR) signaling pathways (Jenwitheesuk et al., 2014). These pathways regulate also normal nervous system ageing and, since age-related neuronal energy deficits would likely contribute to the pathogenesis of several neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease, melatonin could hopefully represent a precious tool to control normal nervous system ageing, neuropathological ageing and longevity.

These studies have led several scientists to suggest various hormone replacement therapies in order to counteract the effects of ageing and i**mprove qua**lity of life.

However, since endocrine and nervous systems are strictly interacting, administering one or more different hormonal molecules could very likely modify the well-tuned systemic homeostasis leading to unpleasant and probably harmful consequences. Besides, there are objective limits to substitution treatment: it is useless to give the body a missing substance if targeted organs have exhausted their functional reserves. So far, at least in some respects, the only effective hormone replacement therapy, by using estrogen and progesterone hormones, is administered in menopausal women. Remarkably, the addition of testosterone to female hormone replacement therapy could apparently contribute to decreasing of the uterine cancer risk and to osteoporosis prevention.

The Immunologic Theory. The Immunologic Theory of ageing, proposed more than 40 years ago by Roy Walford, suggests that the normal process of ageing in man and in animals is pathogenetically related to faulty immunological processes (Effros, 2004; Castelo-Branco & Soveral, 2014; Fulop et al., 2014). As we age, changes in essentially all physiological functions, including immunity, are apparent. The concept of immunosenescence reflects age-related changes in immune responses, both cellular and serological, affecting the process of generating specific responses to foreign and self-antigens. The decline of the immune system with age is reflected in the increased susceptibility to infectious diseases, poorer response to vaccination, increased prevalence of cancer, autoimmune and other chronic diseases. Both innate and adaptive immune responses are affected by the ageing process; however, the adaptive response seems to be more affected by the age-related changes in the immune system. Additionally, aged individuals tend to present a chronic low-grade inflammatory state (sometimes referred to as 'inflamm-aging') that has been implicated in the pathogenesis of many age-related diseases (atherosclerosis, Alzheimer's disease, osteoporosis and diabetes) (Franceschi et al., 2000).

The thymus gland, a central part of the immune system as well as an influential element of the endo-

crine orchestra, produces self-hormones (thymulin, thymosin, thymopentin, and thymus humoral factor) which are participating in the regulation of immune cell transformation and selection, and also synthesizes hormones similar to that of the other endocrine glands such as melatonin, neuropeptides, and insulin, which are transported by the immune cells to the sites of requests (packed transport). Thymic (epithelial and immune) cells also have receptors for hormones which regulate them. This combined organ, which is continuously changing from birth to senescence seems to be a pacemaker of life. This function is basically regulated by the selection of self-responsive thymocytes as their complete destruction helps the development (up to puberty) and their gradual release in case of weakened control (after puberty) causes the erosion of cells and intercellular material, named ageing. This means that during ageing, self-destructive and non-protective immune activities are manifested under the guidance of the involuting thymus, causing the continuous irritation of cells and organs. Possibly the pineal body is the main regulator of the pacemaker, the neonatal removal of which results in atrophy of thymus and wasting disease and its later corrosion causes the insufficiency of thymus. The co-involution of pineal and thymus could determine the ageing and the time of death without external intervention; however, external factors can negatively influence both of them (Csaba, 2016).

Other organs of the immune system undergo involuting phenomena with age: the bone marrow loses its cells due to replacement by adipose tissue, the spleen and lymph glands reduce the number and cellularity of germinal centers. Also T cells decrease their activity over the years: while their number remains relatively unaffected, the proportion of T cells able to perform their function and to proliferate decreases. T helper cells mediated immune response suffers deep changes in elderly: Interleukin-2 (IL-2), a T-cell growth factor, tends to decrease with age, whereas Interleukin-6 (IL-6), which has an inflammatory effect, tends to increase (Kiecolt-Glaser et al., 2003). IL-6 seems to facilitate brain accumulation of amyloid protein, bone resorption and onset of Alzheimer's dementia (Licastro et al., 2003), osteopenia and osteoporosis (Kung Sutherland et al., 2003; Sansoni et al., 2008).

Serum immunoglobulins concentration is also changing with age: both organ specific (anti-endothelium, anti-gastric cells, anti-smooth muscle cells, anti-neuronal cells) and systemic (anti-DNA, anti-mitochondria) auto-antibody increase. Although it was proposed that the age-associated increase in serum autoantibodies reflected a homeostatic function of the immune system that defended the internal milieu by targeting senescent molecules and cells for elimination, recent evidence suggests that autoantibodies may influence the risk of the elderly developing infectious, atherosclerotic, or Alzheimer's disease. Moreover, auto-anti-idiotypic antibodies suppress the antibody response to the nominal antigen and, thus, may contribute to the increased risk of infection and poor response to vaccines in the elderly. In contrast, low levels of autoantibodies to oxidized low-density lipoproteins or to the amyloid beta peptide may contribute to the increased risk of developing atherosclerosis or Alzheimer's disease, respectively (Weksler & Goodhardt, 2002).

Finally, the decreasing activity and loss of efficiency of macrophages and neutrophils, killer cytotoxic cells and natural killer (NK) cells with age could likely explain the increased incidence of elderly specific cancers. The most frequent cancer sites in men over 65 years are represented by lung, colon, rectum, prostate and bladder, whereas the women show higher incidence of tumors in breast, lung, colon-rectum, bladder and pancreas as well as non-Hodgkin lymphoma (Malaguarnera et al., 2010).

Nevertheless, some individuals arrive to advanced ages without any major health problems, referred to as healthy ageing. Studies on healthy centenarians have shown that their immune system maintains a high degree of efficiency: immune cells seem to preserve their ability to respond to antigenic stimulation and to proliferate. The immune system dysfunction seems to be somehow mitigated in this population, probably due to genetic and environmental factors yet to be described (Govindaraju et al., 2015).

Theories of cell damage

Generally, these theories assume that ageing is due to environmental stress and to accumulation of unfavorable external events: different endogenous or exogenous agents (exposure to heat, UV light, ionizing radiation, free radicals, glycosylation mechanisms, mutagenic viruses, microtraumas) could be quite severely harmful to cells, tissues and organs. Nevertheless, such damages can be repaired by numerous physiological mechanisms like antioxidant systems, DNA repair, Heat Shock Protein (HSP) and apoptosis. The repair systems, however, are not able to completely restore the former state, leading to cells, tissues, and targeted organs functional loss. Thus, according to these theories, ageing would

occur as a result of the disequilibrium between damage and repair capabilities.

The Free Radical/Oxidative Stress Theory of Ageing, which was first proposed in 1956, is currently one of the most popular explanations for how ageing occurs at the biochemical/molecular level (Harman, 1956; Bokov et al., 2004; Muller et al., 2007). Free radicals are oxygen compounds with an unpaired electron, resulting from metabolic reactions that, in order to obtain energy from foods, involve oxygen. Free radicals tend to retrieve the missing electron from other cell molecules which, in turn, become unstable and sometimes even toxic. The most common free radicals in aerobic (oxygen breathing) organisms are oxygen free radicals, often referred to as Reactive Oxygen Species (ROS), which include super-oxides (O_2^-), hydroxyl anions (HO^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and peroxynitrite ($OONO^-$). Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal state of cells can cause toxic effects through the production of peroxides and free radicals that damage all cellular components, including proteins, lipids, DNA and mitochondria.

There is considerable indirect evidence supporting the free radical theory of ageing (Knight, 2000). Not only are several major age-associated diseases clearly affected by increased oxidative stress (atherosclerosis, cancer, etc.), but the fact that there are numerous natural protective mechanisms to prevent oxyradical-induced cellular damage speaks loudly that this theory has a key role in ageing [the presence of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, among others; various important intrinsic (uric acid, bilirubin, -SH proteins, glutathione, etc.) and extrinsic (vitamins C, E, carotenoids, flavonoids, etc.) antioxidants; and metal chelating proteins to prevent Fenton and Haber-Weiss chemistry]. In addition, a major part of the free radical theory involves the damaging role of reactive oxygen species and various toxins on mitochondria. These lead to numerous mitochondrial DNA mutations which result in a progressive reduction in energy output, significantly below that needed in body tissues. This can result in various signs of ageing, such as loss of memory, hearing, vision, and stamina. Oxidative stress also inactivates critical enzymes and other proteins. Moreover, in the heart of elder animals cardiomyocytes degeneration and increase of molecules (lipofuscin and hydrogen peroxide) due to free radicals action can be shown. Oxidative stress damage is considered to be involved even in skin ageing (thinning of the epidermis, elastosis, loss of melanocytes associated with an increased paleness and lucency of the skin and a decreased barrier function) as well as neurological degenerative phenomena (Parkinson's disease, Alzheimer's disease, Multiple Sclerosis, Amyotrophic Lateral Sclerosis) (Beal, 2003; Rinnerthaler at al., 2015).

Notably, the reduced free radicals production has also been considered to play a key role in the explanation of caloric restriction (CR) experimental results.

The notion that CR, or the curtailment of energy (food) intake (typically by 20 - 40% of ad libitum consumption) without causing under-nutrition, slows the rate of ageing, prolongs the duration of youthfulness, postpones the onset of age-associated pathologies and extends the life spans of animals of diverse phylogenies (from insects to worms to mammals), has been a leading concept in gerontology for several decades (Austad, 1989; Ramsey et al., 2000; Trepanowski et al., 2011). It is often contended that because of the ubiquity of the association between CR and increase in life span, the mode of CR action may be an evolutionarily conserved “public” mechanism that modulates the intrinsic rate of ageing (Fontana et al., 2010). Indeed, from a theoretical point of view, caloric restriction could delay ageing by slowing/silencing the expression of genes associated with senescence, regardless of what they may be (De Magalhães & Toussaint, 2002).

The conviction that CR has an “anti-ageing” effect, or that it is an antidote to the ageing process, has indeed gained wide popularity: currently, in order to reduce the incidence of old age more common degenerative diseases, main western countries health organizations have suggested dietary programs that associate caloric restriction to daily consume of fruits and vegetables (rich in vitamins and in anti-oxidant properties).

However, emerging evidence disputes some of the primary tenets of this conception. One disparity is that the CR-related increase in longevity is not universal and may not even be shared among different strains of the same species. A further misgiving is that the control animals, fed ad libitum, become overweight and prone to early onset of diseases and death, and thus may not be the ideal control animals for studies concerned with comparisons of longevity. Reexamination of body weight and longevity data from a study involving over 60,000 mice and rats, conducted by a National Institute on Aging-sponsored project, suggests that CR-related increase in life span of specific genotypes is directly related to

the gain in body weight under the ad libitum feeding regimen. Additionally, CR in mammals and "dietary restriction" in organisms such as *Drosophila* are dissimilar phenomena, albeit they are often presented to be the very same. The latter involves a reduction in yeast rather than caloric intake, which is inconsistent with the notion of a common, conserved mechanism of CR action in different species. Although specific mechanisms by which CR affects longevity are not well understood, existing evidence supports the view that CR increases the life span of those particular genotypes that develop energy imbalance owing to ad libitum feeding. In such groups, CR lowers body temperature, rate of metabolism, oxidant production and retards the age-related pro-oxidizing shift in the redox state (Sohal & Forster, 2014).

Glication Theory. Protein modifications such as the non-enzymatic protein glycation are common post-translational modification of proteins resulting from reactions between glucose and the amino groups of proteins. A process of cross-linking reaction of proteins with glucose or its metabolites that occurs with age is known as Maillard reaction (Baynes, 2001). The Maillard reaction involves reaction of amino groups on proteins with aldehydes and ketones to produce advanced glycation end-products (AGEs). The term 'advanced' refers to the fact that AGEs arise through a series of reactive intermediates formed by rearrangement, dehydration, oxidation and fragmentation reactions of carbonyl contained compounds or its adducts to proteins. Many different AGEs have been detected in tissue proteins. They are formed from a wide range of carbohydrates, including glucose, ascorbate, triose-phosphates or methylglyoxal. Interestingly, the AGEs of long-lived proteins such as collagens and cartilage accumulate during normal ageing. They are involved either directly or through interactions with the AGE-receptors in the pathophysiology of numerous age-related diseases including cardiovascular disease, renal disease and neurodegeneration (Soskić et al., 2008; Wagner et al., 2016). As for free radicals, there are physiological mechanisms designed to eliminate the AGE: macrophage can recognize and destroy (by phagocytosis) altered proteins. But, as for other immune system elements, macrophages tend to reduce their number and to lose effectiveness with advancing age, determining an AGEs increase.

The Glication theory suggests that the accumulation of AGEs and the resulting slow cell exhaustion (due to functional and structural cell resources depletion in order to restore the altered proteins) could lead to the inability of cells and tissues to function properly and, finally, to ageing.

However, even if AGEs may contribute to the decline in tissue and organ function with age, especially in age-related chronic disease (such as atherosclerosis, diabetes, arthritis and neurodegenerative disease), the rate of their accumulation in proteins is unlikely to be a primary determinant of the maximum lifespan or rate of ageing of species: they are more probably correlative rather than causative with respect to ageing. Instead, since AGEs are only one of many types of chemical modifications that accumulate in long-lived proteins with age, cumulative damage to the genome as a result of Maillard and other non-enzymatic reactions (such as lipoxidation that produces ALEs, advanced lipoxidation end-products) could likely play a more important role in species lifespan and health in old age.

Hopefully, as the details of the structure and organization of human and animal genomes become available during the next decade, it should be possible to study directly the effects of non-enzymatic reactions on the integrity of the genome. Indeed, inhibition of AGEs/ALEs formation may limit oxidative and inflammatory damage in tissues, retarding the progression of pathophysiology and improve the quality of life during ageing.

The Membrane Theory of Ageing, first described by Professor Imre Zs.-Nagy (Debrechen University, Hungary), stresses that age-related changes of the cells membrane impair its ability to transfer chemicals, heat and electrical processes (Zs.-Nagy, 1978). Indeed, experimental data show that cell membranes become more rigid (less watery and more solid) during ageing. If this involves a decrease of resting potassium permeability, the intracellular potassium concentration will increase. Such an increase is beneficial for the maintenance of cell excitability, however, it represents a drawback for the nuclear functions, since the intracellular ionic strength may reach very high values (even above 400 mEq kg^{-1} cell water), where the chromatin becomes more condensed and the activity of DNA-dependent RNA-polymerase as well as other enzymes probably decreases. Thus, the membrane hypothesis of ageing might explain the decreased protein synthetic activity of old cells, especially of postmitotic ones. Moreover, the membrane structural changes could also lead to lipofuscin (yellow-brown pigment granules composed of lipid-containing residues of lysosomal digestion) toxin accumulation in brain, heart, lungs and skin. Indeed, some of the skin age-pigments referred to as liver or age-spots are composed of lipofuscin. Besides, Alzheimer disease (AD) patients have also

much higher levels of lipofuscin deposits than their healthy controls. Notably, it has recently been supposed that the primary culprit responsible for sporadic AD may be the release of neuronal lipofuscin in the extracellular compartment, an event that should not be harmless since the neuron carries the weight of keeping this substance segregated inside its cytoplasm for decades. This hypothesis considers Amyloid β protein (Aβ) deposition as a downstream phenomenon which is critical and essential but not the absolutely and invariably causative event in determining the onset of AD. It could be that in sporadic AD, Aβ is more than an epiphenomenon because it is tightly linked to the release of lipofuscin in the neuropil, but that the scenario differs when Aβ deposition is induced artificially per se, e.g. in the transgenic mouse model of AD where large quantities of Aβ do not determine significant neuronal degeneration (Giaccone et al., 2011). Lipofuscin is a matrix that recapitulates the insults and damage a neuron receives during the life of an individual. It is therefore likely that its composition and characteristics are influenced by various genetic and environmental factors that may reinforce or weaken each other and, in combination with factors that alter the chance in age-related neuronal death, may modulate the overall individual risk of developing AD. This hypothesis may open up new lines of research in addition to those currently focused on Aβ protein, and thus contribute to more decisive advances in our understanding of the pathogenesis of AD, and in the identification of effective preventive or therapeutic strategies.

In the last decade special attention has been focused on investigating the link among ageing, brain and prion diseases (Kovács & Budka, 2002). Prions ('proteinaceous infectious particles') are unconventional infectious agents consisting of misfolded prion protein (PrP) molecules; in their misshapen state, the molecules aggregate with one another and impose their anomalous structure on benign PrP molecules. Prions thus act as corruptive templates that incite a chain-reaction of PrP misfolding and aggregation. As prions grow, fragment and spread, they perturb the function of the nervous system and ultimately cause the death of the affected individual. The prion diseases in humans include Creutzfeldt-Jakob disease (CJD), Gerstmann–Sträussler-Scheinker disease, fatal insomnia and kuru; in nonhuman species they comprise scrapie, bovine spongiform encephalopathy, chronic wasting disease, transmissible mink encephalopathy and others. Prion diseases are remarkable in that they can be genetic, infectious or sporadic in origin. Infectivity involves the transfer of prions from one organism to another, whereas the genetic and idiopathic cases seem to develop endogenously, owing to the spontaneous misfolding and nucleation of PrP molecules into a self-propagating seed. Recent findings suggest that the 'prion paradigm' - the seeded corruption of otherwise harmless proteins – could also underlie the ontogeny of a widening spectrum of maladies, including common age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Jucker & Walker, 2013). Finally, since PrP molecules are resulting from conversion of a normal, cell-surface glycoprotein (PrP^C) into a conformationally altered (β-sheet enriched) isoform prion, the role of PrP^C in ageing (especially in relation to memory, behavior and myelin maintenance) has been investigated from different perspectives, often leading to contrasting results (Westergard et al., 2007). In ageing, the physiology and the cellular localization of the protein may likely change concomitantly to different biochemical milieus in the cell membrane. Indeed, either membrane composition, in particular lipid raft composition, or additional protein complexes proximity to PrP^C, may influence its physiological functions (Gasperini & Legname, 2014). These changes may have a general relevance for more common causes of dementia such as AD, nevertheless more studies will be necessary to define the precise role of PrP^C in ageing and in the progression of neurodegenerative diseases.

Conclusions and Perspectives

Life expectancy at birth has been increasing for most of the last century in western societies, thanks to the continuous amelioration of medical assistance, to the improvement of the environment (in particular clean, safe water and food), and to the improvement of nutrients. While at the beginning of the 20th century Italian life expectancy was 43 years for women and 42.6 for men (without any relevant variation with respect to past centuries), today the average life expectancy is 80.1 years for men and 84.7 years for women (data from www.istat.it). Similarly, the extreme longevity has been growing in these years. Indeed, the number of centenarians (still in Italy) remarkably increased from 165 in 1951 to more than 15000 in 2011. These results were mainly due to the improvement of medical assistance with respect to dramatic reduction of infectious diseases (which, on turn, dramatically reduced infantile mortality, but also mortality in adult age) and age-related diseases, especially Cardiovascular Diseases and Cancer

(data from www.mortality.org), showing environmental factors strong impact on lifespan and on longevity in humans. While most of the ageing theories are monistic in nature and omit numerous key factors of senescence, ageing and longevity may be multifactorial and very likely modulated by a lucky combination of genetic and non-genetic factors. According to this approach the ageing process could be determined by the sum effects of internal (e.g. genome, immune system, neuroendocrine system, cellular senescence and damage) and external (material, energy, information) factors, although some elements bearing more importance than others (Semsei, 2000). Moreover, epigenetics, modulated by both genetic background and lifestyle, can help to explain the relationship between an individual's genetic background, the environment, ageing and disease: epigenetic modifications could either be a biomarker of the quality of ageing or influence the rate and the quality of ageing (Passarino et al., 2016).

Therefore, investigating the *cause* of ageing has perhaps lead scientists to an impasse, it would have been better to focus on the *main causes* of ageing instead. Because of the high complexity of the human body, where different information systems superpose each other, the cooperation of the elements (counter-effects, regulation) is likely to have the same determining importance of the information level of the unit parts (cells). At the same time, even an answer regarding only one cell type could substantially further contribute to the understanding of the nature of ageing. If searching for genetic and molecular basis of ageing has led to the identification of genes correlated with the maintenance of the cell and of its basic metabolism as the main genetic factors affecting the individual variation of the ageing phenotype, studies on caloric restriction and on the variability of genes associated with nutrient-sensing signaling, have shown that ipocaloric diet and/or a genetically efficient metabolism of nutrients, can modulate lifespan by promoting an efficient maintenance of the cell and of the organism. In this context, the maximal life-span is probably determined by the principle of the weakest element of the chain. All the factors that help to prevent the decrease of the information level of the organism could act against ageing and certain diseases, while the factors which damage the state of the information system could contribute to the acceleration of the ageing process.

Nevertheless, even if, by providing an optimal state for the living system, the maximum life-span of today's people could be substantially lengthened, without changing the present information level of the human body only 'healthy' ageing can be expected.

The human system is not developed enough to live forever. In the end, every living being will always have to come to terms with mortality:

"*Thou know'st 'tis common; all that lives must die, passing through nature to eternity*"

Shakespeare W., Hamlet, act I, scene 2.

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