Progressively accumulating insoluble protein, in the form of a gel with cross-linked random coil chains, is proposed to cause aging by decreasing the water volume available to large colloids and organelles. The decrease in available volume is correlated to the exponential increase with age in mortality rate (Gompertz law).

Key words: aging, cross-linked insoluble protein, Gompertz law, Ogston-Laurent equation.

Introduction

Aging is a complex process leading to a decrease in function and eventual death. Many theories and hypotheses have been proposed to explain aging, such as the action of reactive oxygen species, cross-linking, garbage accumulation, DNA changes, to list a few. In early studies of human nucleus pulposus (NP, central part of the intervertebral disc), the present author found an increase with age in insoluble protein. With the possibility in mind that this may be a general age phenomenon, a theory has been developed that a progressive accumulation of insoluble cross-linked protein is a main cause of aging. This may explain observations linked to aging, e.g. the exponential increase with age in mortality rate, the Gompertz law. This law may be correlated to the Ogston-Laurent equation describing an exponential decrease in volume available to a globular colloid in a randomly coiled polymer network, when the amount of polymer is increased.

In the present survey human chronological aging, as reflected by increasing mortality rates, is the main issue. Aging of cells in vitro is mostly measured as number of divisions (replicative aging) after the famous study by Hayflick and Moorhead, where the lifespan is defined as the maximum possible number of cell divisions. Although the mammalian replicative aging of a cell and the chronological aging of the individual may have some correlation, the replicative lifespan of cells is far from exhausted during the lifetime of the individual.

Basic foundations and possible implications for accumulating insoluble protein being a main cause of aging are outlined.
Nucleus pulposus (NP) as a model of aging

The intervertebral disc fibrocartilage consists of the peripheral fibrous annulus fibrosus (AF) and the central amorphous nucleus pulposus (NP) 12, with a transition zone in between. NP exhibits dramatic changes with age. In childhood it is a colorless gel, in adulthood a slightly yellow-brown tissue with good turgor, in old age brown, without turgor, and high in dry substance, increasing with age 13 (Fig. 1 a). NP lacks blood vessels, contains few cells, and consists mainly of extracellular substances. Moderate amounts of type 2 collagen fibers 14-15 are randomly oriented, which makes it difficult to determine the shrinkage temperature by the usual microscopic procedure 1. It was analyzed by Dickson et al 16 by following the temperature curve at heating.

NP contains large amounts of amorphous non-collagenous protein 1,17,18, a trace of amyloid protein 19 and large amounts of the polysaccharides (glycosaminoglycans) chondroitin-6 sulfate (containing galactosamine and glucuronic acid) 20-22, and keratan sulfate 23 (KS 2, containing glucosamine and galactose). A small amount of hyaluronan (earlier called hyaluronic acid or hyaluronate, containing glucuronic acid and glucosamine) is also present 24. The brown discoloration may be Maillard reaction products of amino acids and sugars, and lipofuscin containing lipid peroxides and proteins.

In an early study of human lumbar intervertebral discs collected at autopsy, the present author 25 found the ratio glucosamine/galactosamine (representing the ratio keratan sulfate/chondroitin-6 sulfate) to increase with increasing age (Fig. 1 b), as confirmed and extended to the cervical and thoracic discs by Scott et al 26 A similar change with age has been described for human hyaline cartilage 27,28, and rabbit NP 29,30.

There is a parallel between the increase with age in dry substance and the ratio glucosamine/galactosamine of AF and NP; in AF there is a rapid increase with age at childhood that in adulthood levels out, whereas in NP there is a continuous increase into old age 1,31. In pathological, dark-brown NP, presumably the remainder of a prolapse 32, a very high ratio glycosamine/galactosamine 1,33 has been found.

The increase with age in dry substance depends to some degree on collagen increase, but mainly on an increase in amorphous non-collagenous protein 1,17,18. Extraction of NP revealed that collagen and most of the non-collagenous protein remains in the residue, and that the ratio glucosamine/galactosamine is higher in the residue than in the extract, i.e. chondroitin sulfate being more soluble than keratan sulfate 34.

Today the biochemistry of the cartilage proteoglycans is known in more detail. In the human NP fibrocartilage and hyaline (nasal, rib, articular) cartilage, the proteoglycan is synthesized as a large complex, (aggrecan), which is capable to aggregate with hyaluronan 35. It is composed of a protein core with chains of chondroitin sulfate chains attached via a galactose-galactose-xylose trisaccharide to a serine hydroxyl; keratan sulfate has another oligosaccharide linkage to a threonine or serine hydroxyl. The head part (N-terminal end) of the protein core attaches, together with a small link protein, non-covalently to a hyaluronan chain, presumably intertwined with collagen fibers. The keratan sulfate chains are mainly located near the head part of the protein core, whereas the chondroitin sulfate chains are mainly located towards the tail of the core 37,38.

NP also contains smaller proteoglycan components unable to aggregate with hyaluronan 37,38. By studying NP proteoglycan synthesis in vitro with 35S labelling in the
presence of proteolysis inhibition, Oegema\textsuperscript{39} showed that only the large proteoglycan (aggrecan) was synthesized. This implies that the observed smaller proteoglycan components unable to aggregate with hyaluronan are secondary split products. As shown for articular cartilage, degradation of the aggrecan proteoglycan starts with scission of the protein core\textsuperscript{40}. After scission, the head part of the proteoglycan core, with most of the keratan sulfate chains remain in the insoluble fraction, tethered to the hyaluronan-collagen residue, whereas the chondroitin sulfate chains bound to the liberated part of the protein core, are in the soluble fraction.

This provides a rationale to the increase with age in the ratio glucosamine/galactosamine (keratan sulfate/chondroitin sulfate)\textsuperscript{25,26}, its parallel with dry substance increase\textsuperscript{1}, increase in non-collagenous protein\textsuperscript{1,17,18}, higher extractability of galactosamine (chondroitin sulfate) as compared to glucosamine (keratan sulfate)\textsuperscript{34}, and the constant ratio at different ages between hyaluronan and keratan sulfate\textsuperscript{26}. If the concentration of chondroitin sulfate is regarded as a rather constant steady state of synthesis and degradation, and non-degraded insoluble keratan sulfate is continually accumulating, the increase with age in the ratio glucosamine/galactosamine may be considered as a measure of the increase with age in insoluble protein. This may be reconciled with the parallel between the increase with age in dry substance and ratio glucosamine/galactosamine\textsuperscript{1}. That this may be an important factor in aging is indicated by individuals with a high ratio glucosamine/galactosamine tend to have succumbed from heart attacks (Fig. 1 b).

**Exclusion effects; Ogston-Laurent equation**

A globular colloid at equilibrium in a random coil polymer solution is excluded from part of the water volume, since many locations are too small to lodge the colloid. Ogston\textsuperscript{41} derived mathematically and Laurent et al.\textsuperscript{42-44} proved experimentally the relation between the fraction of the volume available ($K_{av}$) to a colloid and the concentration of the polymer:

$$K_{av} = \exp \left[ -\pi L (r_s + r_i)^2 \right]$$

where $\pi = 3.14...$, $r_s$ radius (Stokes’) of the colloid and $r_i$ radius of the polymer chain. The equation describes an exponential decrease in $K_{av}$ versus a linear increase in $L$ (Fig. 2a), whereas the logarithm of $K_{av}$ is a linearly related to $L$ (Fig. 2b):

$$\ln K_{av} = -\pi L \left( r_s r_i \right)^2$$

Ogston\textsuperscript{41,45} also derived the relation between the root-mean-square value ($R$) of the radii of the spaces within the network and the concentration of the polymer (Fig. 2c):

$$R = (\pi L)^{-\frac{1}{6}}$$

where $R$ is the nominal value, the effective value being ($R - r_i$). This equation was supported experimentally by White\textsuperscript{46}. Substitution in Eq.1 gives the Ogston-Laurent equation as

$$K_{av} = \exp\left[ -\left( r_s + r_i \right) / R \right]^2$$

thus, $K_{av}$ being related to the quotient ($r_s + r_i$) / ($R - r_i$)

That $K_{av}$ of a globular colloid decreases with increasing polymer concentration means that its **effective** concentration increases, leading to an increase in colloid osmotic pressure\textsuperscript{47}. 
The Ogston-Laurent Eq.1 may be assumed to be valid in vivo. This has been supported experimentally by equilibrating labeled polyethylene glycol (2 kDa, \( r_s = 32 \) Å) and mannitol (\( r_s = 4 \) Å) in nephrectomized rabbits. Comparison between the \( K_{av} \) of polyethylene glycol in extracellular water (mannitol-space), and polysaccharide content of various connective tissues exhibited a reasonable parallel between calculated and experimental results.

\( K_{av} \) in Eqs.1 and 4 refers to a system at equilibrium. However, \( K_{av} \) may be applied also to a system at non-equilibrium, with the globular colloid moving in the random polymer network. According to the Delesse principle, the area available for passage of a globular colloid through a random coil polymer network is the same as the volume available at equilibrium. By meticulously comparing the mobility retardation \( (m/m_o) \) of proteins in polyacrylamide gel electrophoresis with their \( K_{av} \) as determined by size-exclusion (gel) chromatography, Morris and Morris found the relation

\[
m/m_o = a K_{av} + b
\]

Eq.5

where \( m \) is the mobility in polyacrylamide gel, \( m_o \) the mobility at extrapolated zero polymer concentration. \( a \) is a factor slightly below unity, \( b \) a term slightly above zero, \( (a + b) = 1 \). Fig. 2d shows a graphic representation of Eq.5. It seems that \( m/m_o \) is slightly higher than \( K_{av} \) indicating an extra space available for mobility. This may be reconciled with the observation that the value of \( b \) was inversely related to the degree of cross-linking. It seems reasonable to assume that the moving colloid may nudge a polymer chain a little aside, thus increasing the possibility of the colloid pass, and that the polymer compliance may decrease when the distance between the cross-links increases.

Another mobility equation was empirically found by Laurent et al. As slightly modified by Ogston et al., it may be formulated

\[
m/m_o = k \exp \left[ - (\pi L)^{1/2} (r_s + r_o) \right]
\]

Eq.6

where the empirical constant \( k \) is slightly above unity for small colloids, but increases to two or more for very large colloids. The value of \( k \) may be calculated by comparing the value of \( m/m_o \) obtained by Eq.5.

**Gompertz law**

By studying human life-span tables, Gompertz found the number of survivals to be linearly related to the age at death as a second order exponent. The law is usually expressed as a relation between the rate of mortality \( (R_m) \) and age:

\[
R_m = R_o e^{\alpha x}
\]

Eq.7

where \( R_m \) (more exactly hazard rate) is the rate of mortality at age \( t \), \( R_o \) mortality rate at start of observation (usually extrapolated to birth), \( \alpha \) Gompertz slope (assumed to be a measure of aging rate), and \( x \) age at death, usually in years. \( R_m \) increases exponentially with age (Fig. 3a). The equation has reached a wide acceptance. It is often written in logarithmic form (Fig. 3b), showing a linear relation between the logarithm of \( R_m \) and age:

\[
\ln R_m = \alpha t + \ln R_o
\]

Eq.8

From this equation the values of the slope \( \alpha \) and \( R_o \) are determined.
Integration of Eq. (7) gives the fraction of survivals (S), the ratio between number survivals living at age x (Fig. 3c):

$$ S = \exp \left[ \left( \frac{R_o}{\alpha} \right) (1- \exp \alpha x) \right] $$

Eq.9

The number fraction deceased (N) versus age obtained by combining Eqs.5 and 7 exhibits a Poison distribution (Fig. 3 d):

$$ N = R_m x S $$

Eq.10

Makeham \(^{57,53,55,58}\) found an improved fit to observed mortality data by addition of a constant A representing the contribution of extrinsic causes to mortality:

$$ R_m = R_o e^{\alpha x} + A $$

Eq.11

A may be considered as the risk of succumbing to an external cause of mortality, usually assumed to be approximately constant during lifetime. For total mortality risk (R_m), the risk of mortality because of age-related frailty has to be added (c.f. mortality of pedestrians in road accidents \(^{53}\)).

The remarkable increase in human lifespan since ancient times depends on a decrease in mortality of extrinsic causes (Makeham’s component A), whereas the aging rate, represented by the Gompertz a, presumably has been rather constant. In modern western countries intrinsic causes of mortality predominate, implying that in order to prolong the lifespan further, the rate of aging has to be slowed down.

The Gompertz law, referring to intrinsic mortality, has been applied with neglect of A. Strehler and Mildvan \(^{59,54}\) described the relation

$$ \ln R_o = -73.4 \alpha - 2.66 $$

Eq.12

which has been claimed to show that the rate of aging (\(\alpha\)) decreases in harsh conditions (high \(R_o\)). However, Gavrilov and Gavrilova \(^7\) showed this to be a mathematical artefact. By application of the Gompertz law neglecting A, the value of \(\alpha\) and \(R_o\) becomes too low and too high, respectively. There is a relationship between the values of \(R_o\) and neglect of A, and a high \(R_o\) is a sensitive indicator of A - neglect. Despite considering A, a similar relation was described by Gavrilov and Gavrilova \(^7\), the compensation law of mortality:

$$ \ln R_o = -95\alpha - 0.67 $$

Eq.13

where 95 was stated to represents human species specific lifespan, and – 0.67 corresponding to ln.0.51, 0.51 being the human species specific \(R_m\). However, application of this equation also includes elevated values of \(R_o\), indicating some residual neglected A \(^{58,60}\). Furthermore, the last decades these relations has disappeared \(^{61,62}\), since the increase in lifespan reflects a decrease in the value of \(R_o\), whereas the value of \(\alpha\) has been constant.

Carnes et al \(^{63}\) studied the mortality rate of a U.S – cohort, excluding causes of mortality unrelated to aging. The unbiased Gompertz law (Eqs.7,8) was found to be followed in the age-range 15-90 years. As judged from their figures, the values of \(\alpha\) and \(R_o\) were about 0.1 and 1.6 x 10^{-5} for males, and 0.1 and 1.3x10^{-5} year^{-1} for females, respectively \(^{58}\).

It has been claimed by several investigators that at a very high age the value of \(\alpha\) decreases almost to zero, implying a slowing down of the aging rate. However, as found by Gavrilov and Gavrilova \(^{64}\), this is largely a mathematical artifact depending on an exponential increase at this age in \(R_m\) (hazard rate) within the one year interval applied. By instead applying one
month intervals, a $\alpha$—value of centenarians (up to 105 years of age) was found to be only slightly lowered (to about 0.0085 as judged from their figures $^{64}$).

As expected the Swedish population largely followed the unbiased Gompertz law (Fig.4). However, to use $\alpha$ as a measure of aging rate, not only mortality unrelated to aging has to be excluded, the cohort analyzed has also to be homogeneous. By studying the number of deaths at different ages of obituary notices in the Stockholm area, assumed to represent a cohort with optimal living conditions, it was found that especially the male cohort is inhomogeneous; there was a peak in number of deaths around 65 years of age (not shown). This peak, absent in the unbiased Gompertz curve (Fig. 3 d) was confirmed by official statistics of whole Sweden $^{65}$ (Fig.4 d). This extra peak shows that the Swedish male cohort is inhomogeneous, and cannot be used to determine the unbiased Gompertz parameters. The female cohort (Fig. 4d) has a much less pronounced 65-year-peak. Thus, to determine the nearest possible unbiased Gompertz parameters of the Swedish population the female data should be used. The female values of $\alpha$ and $R_m$ were found to be about 0.1 and $1 \times 10^{-5}$ year $^{-1}$, respectively (Fig.4d), in fair agreement with Carnes data $^{63}$. The $\alpha$—value 0.1 means a doubling of $R_m$ after about 7 years ($\ln 2/\alpha = 6.93$). It is interesting to speculate that the extra male peak at 65 years of age may at least partly be ascribed to Y-chromosome haptogroup 1, common in Scandinavia, predisposing to early heart attacks $^{66}$.

As shown by Jones $^{67}$, the exponential increase with age in mortality rate according the Gompertz law may be applicable also to specific causes of mortality (see later).

**Cross-linking of proteins**

Boeseken $^{68}$ and Verzàr $^{69,70}$ proposed that cross-linking of proteins may be a cause of aging. Verzàr studied extracellular aging by measuring the tensile strength on heating tendons from old animals. The strength was found to be increased in old tissues, whereas the shrinkage temperature of collagen was not increased, indicating that cross-linking within the collagen triple helix chains was not involved. Treatment of the tendon with trypsin was found to abolish the age-related increase in tendon tensile strength $^{71}$, indicating that the tensile strength effect depends on proteins outside of trypsin resistant collagen triple helix.

There are several possibilities for protein cross-linking. Cerami $^{72}$ found that aldehyde carbonyl groups of open chain glucose could by complex reactions form cross-links between protein amino groups. This may change the conformation of the protein, which may expose e.g. $-\text{SH}$ groups to form $S-S$ linkages, as studied in lenses of the eye $^{73}$.

Harham $^{74,75}$ introduced reactive oxygen species, mainly formed by mitochondria, as a cause of aging by initiating deleterious chain reactions $^{76}$ in components, such as DNA and lipids. In proteins there are several reactions $^{77,78}$ that may lead to cross-linking.

Red blood corpuscle, lacking both mitochondria and nucleus has in the human a lifespan of only about 120 days. It has been proposed to be a correlation between the lifespan of mammalian species and the lifespan of their erythrocytes $^{79}$. The destruction of the erythrocyte is initiated by cross-linking of a membrane protein $^{79}$.

As will be discussed later, it is assumed that cross-linking of proteins leads to the formation of insoluble protein.
**Theory proposed: Insoluble protein accumulation a main cause of aging**

Mainly through the formation of cross-links there is a progressive accumulation of insoluble protein forming a gel with cross-linked random coil polypeptide chains. This network excludes large colloids and organelles from part of the water volume. In this system the Ogston-Laurent relation is valid. It is assumed to be a correlation between the exponential increase with age in $R_m$ according to the Gompertz law (Fig.3a), and the exponential decrease in volume available ($K_{ov}$) to a large colloid with a linear increase in concentration of the cross-linked insoluble protein (Fig.2a). Thus, the Gompertz slope $\alpha$ becomes a measure of the rate of increase in amount of insoluble protein.

Small molecules involved in intermediary metabolism are very little excluded by a polymer network (see Fig.2a). This may be reconciled with the basal metabolism being very little affected by aging, as shown in a Strehler's figure 54. Moderately large colloids may be slightly excluded, leading to a moderate, within the lifespan almost linear decrease in $K_{ov}$ that may correlate with a slight, within the lifespan of the individual approximately linear decrease with age of many physiological functions 54.

The transport along the microtubule chains seems not to be seriously affected by the insoluble protein fraction. As mentioned above, the basal metabolic rate, a measure of mitochondrial oxygen consumption, is only little affected by aging as compared to other physiological functions and mortality rate 54. This may seem as a contradiction to the proposed theory of insoluble protein as a cause of aging, since a large organelles, such as mitochondria, should be very sensitive to a decrease in $K_{ov}$. However, the microtubules are synthesized in parallel rows. In such a system the Ogston-Laurent equation (Eq. 1) is not valid, since it requires the polymer to be in a random orientation. Along the parallel microtubule polymer chains, mitochondria 80 and transport vesicles may move rather unhindered, as in tunnels. Although vesicles are transported bound to microtubule chains, the mitochondria are detached from the microtubules when the local concentration of ATP has reached a high concentration 81. The mitochondria then presumably follow fluid movements to locations within the cytoplasm high in osmotic pressure and with high concentrations of phosphate ions and pyruvic acid 82.

The parallel between total mortality rate increase with age, and the mortality rate increase of specified diseases found by Jones 67 suggests a common unifying mechanism, here proposed to be accumulating insoluble protein. Possible mechanisms will be outlined for atherosclerotic disease (intercellular process) and cancer (intracellular process), the major intrinsic causes of human mortality. The analysis will be limited to the age range 40–80 year. At lower age, age-related mortality is rare. At higher age there may be more than one disease contributing to mortality, and small different cohorts may prevail.

**A model of fatty streak and atherosclerosis development**

The development of atherosclerosis may be schematically divided into three steps. The first is the formation of fatty streaks, which is reversible. The most studied second step is an inflammatory reaction involving inflammatory cells, fibroblasts and smooth muscle cells creating an atherosclerotic plaque, eventually calcified. In the third step, the formation of an arterial thrombus on a rupturing plaque, there may be serious clinical symptoms, e.g. a heart
infarct. There are several risk factors for developing atherosclerotic diseases, such as hypertension, high blood lipids, diabetes, smoking, stress and, most importantly, aging.

The precipitation of lipids in the arterial intima is a necessary initial step for the formation of an atherosclerotic plaque. It seems logical that an increased level of blood lipids should promote the deposition in the arterial intima of lipids, and high levels of low density lipoprotein (LDL) has in several studies been shown to predispose to atherosclerotic disease. It is known that if the blood is low in lipids, atherosclerotic development is absent, even in the presence of risk factors promoting the second and third steps of atherosclerotic disease.

An increase in blood pressure may be expected to increase the entrance of plasma containing lipids into the arterial intima. The importance of blood pressure may explain why plaques are preferentially formed in central arteries, where the blood pressure is high, rather than in peripheral smaller arteries, where the blood pressure is lower. In veins the blood pressure is near zero, and atherosclerosis is not observed. However, a vein transplanted to an arterial position may develop atherosclerotic plaques.

To enter the arterial intima, lipids have to pass the endothelial cell barrier. Removal of endothelial cells is a way of experimentally producing fatty streaks. The passage through the endothelial cells may occur by endocytosis, or by passage through channels between the cells, the last mechanism presumably prevailing. Smaller lipid particles seem to pass easier through the narrow channels.

Atherosclerotic plaques are preferentially formed at arterial branch points and around costal artery orifices, where turbulence of the blood may develop. This may lead to shear stress of endothelial cells, which may increase their permeability of e.g. lipids.

Microalbuminuria (increased amount of albumin in urine) is an independent risk factor for developing atherosclerotic diseases. It is caused by increased leakage through the glomerular endothelium. A similar mechanism in arterial endothelial cells, possibly due to some noxious agent, may promote the passage of lipids into the intima, explaining why microalbuminuria is an independent predictor of atherosclerotic disease.

It is assumed that in arterial intimal extracellular substance there is an increase with age in insoluble protein, similar to NP. When the insoluble protein content increases during aging, the $K_w$ of the lipoproteins decreases. This is proposed to explain the exponential increase with age in heart attack mortality. In the human precipitation of lipid may also be promoted by an affinity between the proteoglycan and the lipid.

The proposed model may explain not only the exponential increase with age in atherosclerotic diseases, but also why hypertension, high blood lipids and disturbances of the intimal endothelial cell layer may promote atherosclerosis.

**Insoluble protein accumulation and cancer development**

Cancer may be induced by congenital disposition or environmental influence (radiation, chemicals, virus), but seems in the old to be mostly spontaneous. The incidence of various cancers may vary between different geographic locations and various environmental factors, but total cancer mortality seems to increase with age in an exponential manner. There is a parallel between cancer cell development and evolution. Evolution is believed to be promoted by rearrangement of chromosomes and point mutations. Cancer cells,
developing according to Darwinian principles, often have chromosomal aberrations, as found by Rowley\textsuperscript{85}, such as aneuploidy (wrong number of chromosomes), translocations (exchanges between chromosomes), deletions or inversions. It is here assumed that chromosomal aberration is an important cause of non-familiar cancer development at adult and old age. An increase with age in insoluble protein causing an age-related exponential decrease in available volume (decrease in $K_{av}$, see above) may promote cancer development by increasing the possibilities of chromosomal aberrations. Accumulated insoluble protein may also disturb centromere function, which may contribute to cancer development\textsuperscript{85,86}.

An interesting model of cancer development has been proposed, where the old budding yeast cell was a model of a cancer stem cell\textsuperscript{88}. A yeast cell undergoes an asymmetric division to produce a small daughter cell. This may be repeated about twenty times, when the mother cell has reached its replicative lifespan. This is assumed to depend on the accumulation of insoluble protein\textsuperscript{88,89} (there is no telomere shortening at yeast cell divisions). The old mother yeast cell develops serious chromosomal aberrations\textsuperscript{90}, which has been proposed to be secondary to accumulating insoluble protein\textsuperscript{88,89}. That the DNA-changes are secondary rather than primary effects is supported by the observation that daughter cells from spores of old cells give daughter cells with the same viability as spores from young cells\textsuperscript{92}.

Cancer develops in tissues with mitotic activity and is usually considered to evolve from a single cancer stem cell by a number of mutations. Tomasetti and Vogelstein\textsuperscript{93} found a correlation between cancer risk and number of stem cell divisions in different tissues. Stem cells seem to be able to undergo an indefinite number of divisions. This means that if accumulation of insoluble protein is a cause of aging, the accumulation does not involve protein synthesis at mitosis. The accumulation of insoluble protein leading to chromosomal aberrations must evolve between the mitoses, where time is an important factor. This may be reconciled with major human cancers, such as the prostate of male and breast of females, evolving from slowly growing tissues with long time between the mitoses.

Taken together the scenario evolves that sooner or later, by a stochastic event in a stem cell, some chromosomal aberration starts a transformation of a normal stem cell into a precancerous stem cell. The chance for this to occur may increase with age due to the accumulated insoluble protein. The picture is somewhat disturbed by the fact that cancer mortality rate may deviate slightly from the Gompertz law (see below).

**Discussion**

The presented data show that there is a parallel between the unbiased Gompertz law describing an exponential increase with age in mortality rate ($R_m$ or hazard rate), and the Ogston-Laurent equation describing an exponential decrease in available volume ($K_{av}$) to a global colloid in a polymer solution, when the concentration of the polymer is increased. It is proposed that this reflects an increase with age of insoluble cross-linked protein chains forming a random network excluding large colloids and organelles from part of the water volume. Age ($x$) then becomes a measure of polymer (insoluble protein) concentration ($L$), and Gompertz slope ($\alpha$, aging rate) of the rate of increase in $L$.

In mathematical terms, in an aging system the rate of failure increases with time. Weibull\textsuperscript{94} described the rate of breaking down of mechanical devices, where it is assumed that the
failure may depend on breaking of several parts. When applied to human mortality \( R_m \) is related to age raised to \( e.g. \) the fourth power \( (x^4) \), referring to a system with five weak points that may break.

\[
R_m = x^4 + A
\]

Eq.14

In a Weibull model there is a linear relation between the logarithm of number of breakages (here \( R_m \) or hazard rate) and the logarithm of age. Such a relation has been found for the increase with age in incidence of some cancers \(^{80}\). According to the Gompertz law there is a linear relation between the logarithm of \( R_m \) and age, the Gompertz exponential increase in mortality with age being exaggerated in cancer. Although accumulation of insoluble protein may be one cause of increase with age in cancer mortality, there may be several other causes, such as genetic disposition, point mutations, virus, radiation, which may contribute towards a Weibull type of mathematics.

There are many events happening in a living organism during the passage of time. Intracellularly there is a slow accumulation of insoluble lipofuscin, consisting of cross-linked protein and peroxidized unsaturated lipids, originating from lysosomal degradation of mitochondria and other organelle membranes. The cell cannot get rid of lipofuscin, and its accumulation has been proposed to be a cause of aging \(^{95}\). However, lipofuscin is compact and has a small exclusion effect, its own volume only, and presumably with a very small contribution to mortality before 80 years of age.

Of greater concern is proteins accumulating as insoluble amyloid beta fleet fibrils, \( e.g. \) in the Alzheimer disease. Amyloid may be formed by many proteins. Although even \( NP \) contains a small amount of amyloid \(^{19}\), the amount is normally small in the absence of a genetic predisposition, presumably with a limited contribution to mortality statistics before 80 years of age. Accumulating insoluble protein may hinder transport of precursor oligomers and promoting fibril precipitation, thus contributing to the exponential increase with age in dementia incidence.

Shortening of telomeres protecting chromosome ends at cell divisions has been proposed to be linked to aging. Shortening of telomeres in the absence of telomerase is a measure of number of divisions of the cell, which does not necessarily mean that it is a primary cause of aging. Most of the human aging processes occur post-mitotic in non-dividing cells. It may be assumed that yeast cells have similar causes of aging as the human organism, and yeast cells divide and age without telomere shortening. Mice have very long telomeres, and telomere shortening cannot explain the short lifespan and short number of possible divisions in fibroblasts of these animals.

Accumulation of insoluble protein may be assumed to be the sum of accumulation during mitosis and accumulation after the mitosis. Modern cells seem to be able to divide indefinitely provided there is no telomere shortening. This implies that the insoluble protein accumulation is postmitotic. This may be reconciled with the observation \(^{54}\) that the replicative lifespan of fibroblasts decreased when the time between mitoses increased.

At cell division there is a dilution of insoluble matter. This may have a rejuvenating effect, and it seems that aging is slow or absent during growth. Lansing \(^{96}\) proposed that aging starts when cells stop dividing. This is supported by the observation that constantly growing female flounder have a long lifespan, whereas male flounders that stop growing have a much shorter lifespan \(^6\).
Accumulation of insoluble protein may cause frailty of the old in many ways. It may cause stiffness of the heart muscle, resulting in a decrease in ejection fraction. Stiffness of arteries may cause an increase with age in systolic pressure. At high age the breathing may be inadequate, leading to shortage of oxygen. The food consumption may decrease, which may lead to insufficient supply of essential nutrients. The vesicular transport of GLUT 4 may be compromised, which may contribute to the development of insulin resistance and diabetes type 2. Decrease in water intake may lead to an increase in insoluble protein concentration and a decrease in $K_{av}$.

The protein synthesis of ribosomes should be disturbed by the presence of an insoluble protein fraction. This may have far reaching consequences. A decrease in protein synthesis at old age may contribute to senile sarcopenia of muscles, osteoarthritis (arthrosis) of joints, and osteoporosis in bone, and possibly to the negative nitrogen balance of the old. Enzymic activities involved in carbohydrate lipid, protein and epigenic metabolism may be compromised.

There are several changes with age not leading to death that may be ascribed to an accumulating insoluble protein fraction. This may cause a decrease in the turgor of skin (cf. NP). The graying of hair, usually ascribed to enzymic failure, may depend on hindrance of the complicated transport of melanin from the melanocyte to the hair follicle. The incidence of Down’s syndrome (chromosome 21-trisomi) increases in an exponential manner with the age of the mother; with increasing age of the oocyte the effective concentration of the chromosomes increases, which increases the risk of chromosomal aberrations. In cell culture of fibroblasts there is a delated start of cell divisions of old cells, but then the cells divide in the same rate as young cells; the delated start may be ascribed to the insoluble protein fraction, which is diluted after the initial cell divisions.

There are several experimental methods to delay aging and prolong lifespan. The first and still best method is caloric restriction, which was shown to prolong lifespan of mice and rats; the animals grow slower, became smaller, and the bones became brittle. It seems that in caloric restriction there is an increase in protein autophagy, which decreases the amount of misfolded proteins available for cross-linking. In addition the metabolic pattern is changed away from pathways promoting the building up of structures not acutely needed for keeping alive. Other lifespan-prolonging methods hinder the IGF-1 and mTorc-1 pathways, which may have similar effects as caloric restriction.

According to the protein accumulation theory, the best method to retard aging would be to thwart cross-linking reactions. That cross-linking is important in aging is indicated by agents blocking cross-linking carbonyl groups, such as aminoguanidine and guanosine, have a rejuvenating effect. The age-promoting reactions seem to be non-enzymatic of various types, presumably different at different locations.

Although the evidence is circumstantial and many details remain to be explored, exclusion effects of metabolically inert cross-linked protein may explain several phenomena of aging. The progressive accumulation of insoluble protein concept also fulfills Strehler’s criteria of an age theory by being universal, intrinsic, progressive and deleterious.

Acknowledgements

The studies on human nucleus pulposus were performed at the medical chemistry department 2 (head: Erik Jorpes) of Karolinska institutet, and on connective tissue
extracellular exclusion effects at the medical chemistry department (head: Torvard. C. Laurent) of Uppsala University. Valuable discussions and support is greatly appreciated.

Start date: 2016-07-03

References and notes

6. Hayflick L. How and Why We Age. Ballantine Books, New York 1994, 1996. – p. 21 female flounder grows indefinitely and does not show age changes, but the male reaches a fixed size and ages. – 47 changes with age of protein is a problem in the eye’s lens, but “these same protein changes occurring elsewhere in the body produce no discomfort” (which is contrary to the proposal of the present paper). – 116-124 limit of replication of normal cells.
7. Gavrilov LA, Gavrilova NS. The Biology of Life Span; a quantitative approach. Harwood academic (1991) – 41 calculation of hazard rate. – 46 the Weibull breaking down law – 50-61 the Gompertz-Makeham law. – 141 -148 the Strehler-Mildvan correlation. – 148-156 compensation effect of mortality. – 207 on the basis that fish at hypothermia live longer and grow larger, it is proposed that cellular activities based mainly on enzymatic activity has a low activation activity and thus are rather insensitive to variation in temperature, whereas aging mainly depends on non-enzymatic reactions with high activation energies and sensitive to temperature. – 212-224 cellular replicative life span reviewed.
12. Luschka Die Halbgelenke der menschlichen Wirbelsäule,
15. Eyre DR, Muir H. Types I and II collagens in intervertebral disc. Biochem J. 157 (1976) 267-70. -- Collagen type 2 predominates in NP, whereas type 1 predominates in AF. In the transition zone there is a mixture of both.


22. Meyer K. -- Chondroitin-6 sulfate was isolated and characterized from a chorda dorsalis tumor.


27. Kuhn R, Leppelmann HJ. Ann 611 (1958) 254-


29. Davidson EA, Woodhall B.

30. Hallén A. Application of ion exchange chromatography to the study of connective tissue glycosaminoglycans. Abstracts of Uppsala dissertations from the faculty of medicine 204 (1974). -- Connective tissues from nephrectomized rabbits equilibrated with $^{14}$C labelled polyethylene glycol ($r_s = 32$ Å) and $^{14}$C labelled mannose ($r_s = 4$ Å) were digested with papain, put on top of a Sephadex G50 gel filtration column on top of a DEAE-cellulose column (Hallén A. J. Chromatogr. (1974) 71: 83-91). In the high molecular weight fraction, polyethylene glycol was recovered in the void volume. The polysaccharides were separated by elution from the DEAE-cellulose column at 60°C with a litium chloride gradient. In the low molecular weight fraction of the Sephadex column, mannose was recovered, used as a measure of the extracellular water volume. The radioactivities of the tissues were related to the value in serum. -- In analyses of the polysaccharides, the NP chondroitin sulfate was partly separated from keratin sulfate. and the ratio keratan sulfate/chondroitin sulfate was higher in old rabbits than in young. In aorta three
components were observed corresponding to hyaluronan, heparan sulfate and chondroitin-sulfate--dermatan sulfate.

31. At infancy the peripheral AF is extensively supported by blood capillaries, which may be reconciled with a high rate of insoluble protein synthesis at infancy. In the adult NP gets nutrition mainly from the center of vertebrae, that may result in a continuous synthesis of insoluble protein. Nutrition of NP may also be linked to day and night variation of pressure; at day’s upright position water is pressed out, at night’s sleep imbibed.

32. NP may prolapse towards the vertebral channel and compress spinal nerves. In the lumbar region this may cause sciatica, pain may be caused by proteoglycan-nerve contact. Prolapses may also occur into the vertebrae (Schmorl’s nodules). Where the prolapse happens there is a bleeding and inflammatory reaction, and presumably a rich formation of blood capillaries, which may accelerate protein synthesis and age changes. Occasionally the age changes of the intervertebral disc are called disc degeneration and implied as a cause of prolapses. However, the increase with age of keratan sulfate/chondroitin sulfate and insoluble protein is presumably not a cause of the prolapse. The prolapse is rather an effect of high pressure and physical shear forces within the disc. The shear forces are most pronounced at the rear part of the disc in neck and lumbar (lordotic) regions, where the volume of the disc is decreased.


34. Hallén A. Extraction of mucopolysaccharides from connective tissue. *Acta Chem. Scand.* 14 (1960) 1828-32. – In tissues where hyaluronan is a major component, such as umbilical cord and skin, it is extractable. This is in contrast to cartilaginous tissues, where hyaluronan is a minor component and resistant to extraction.


Eq. 6 was proposed, based on Einstein’s (1905) unit step (\(\lambda\), root mean square value) model of Brownian movement, where the distance traveled is linearly related to \(\lambda^2\). However, the diameter of the space within the polymer network (root mean square of a distance) was related to \(\lambda\) rather than to \(\lambda^2\), which leads to Eq. 6. As expected, Eq. 6 could not be based on Einstein’s (1908) ‘phenomenological’ model of Brownian movement. – A small contribution to the value of \(k\) in Eq. 6 may be movement in the ultracentrifuge gravitational field of diluted polymer networks.

46. White ML. The permeability of an acrylamide polymer gel. J. Phys. Chem. 64 (1960) 1563-5. – The author, who was not aware of Ogston’s equations, studied water permeability at increased pressure in polyacrylamide gels with different concentrations and calculated the poor size based on the Poiseuille law. The relation between \(R\) and gel concentration was similar as calculated from Eq. 2 (Fig. 2c).


52. Gompertz B. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Phil. Trans. Roy. Soc (London) A 115 (1825) 513-85. – The law may also be expressed as \(R_m = -1/n \frac{dn}{dx}\), where \(n\) is the number of survivals at age \(x\).

53. Comfort A. The Biology of Senescence, 3rd edition. Churchill Livingstone U.S.A. (1979). – 23 Gompertz law. – 25, Fig. 1.3. Distribution by age of total mortality and mortality of pedestrians in road accidents are very similar. – 195 – 197 In cell culture the time lag in reaching the mitotic peak lag increases with age.

54. Strehler BL. Time, cells, and aging, 2nd edition. Academic Press, New York (1977) – 13 the criteria for a aging theory, e.g. universality, is discussed. – 43 the duration of time under culture conditions rather than the total number of prior cell divisions which limits division potential. – 59 senescence starts when growth ceases. – 65 experiments (Sonneborn et al.) with Paramecium indicates that age changes in the cytoplasm causes chromosomal aberrations. – 103- mortality rates and Gompertz law is exemplified and discussed. The author is unaware of the biasing effect of the Makeham contribution to mortality unrelated to aging. – 112 Fig. V-7 (based on Shock et al.) shows how various functional capacities decrease with age. The basal metabolism decreases by only about 1% per year, in parallel with a decrease in cellular water. – 127 Fig VI-I shows a Gompertz plots for cause-specific mortality rates; the cancer line is to low,

56. Hazard rate is the proper unit to be used. It is usually calculated with the simplified Sacher expression, \( \ln (1 - q_x) \), where \( q_x \) here represents \( R_m \), the risk of dying within a specified age-year. It is assumed that \( R_m \) is constant during the year measured (not true for centenarians, where it increases). Since hazard rate is practically identical to \( R_m \) up to 80-90 years of age (\( q_x = R_m \) at 90 years is 0.08), \( R_m \) may be considered synonymous with hazard rate up to this age.


58. Hallén A. Makeham’s addition to the Gompertz law re-evaluated. Biogeront.10 (2009) 517-22. – The Makeham term may be considered as the risk of mortality at young age from a cause of mortality unrelated to aging, assuming that this risk is approximately constant during the lifetime. At higher age the total mortality risk includes the risk of dying from a age-related cause; a special impact may be more deleterious because of the frailty at higher age (cf. Comfort 53 on pedestrians road accidents).


60. An alternative definition of species specific lifespan could be the age at extrapolated \( R_m = \) unity, which in the human is at 108 - 110 years of age.

61. Finch


64. Gavrilov LA, Gavrilova NS.

65. Statistiska Centralbyrån


68. Boeseken

69. Verzár

70. Verzar


72. Cerami

73. The age changes of the eye’s lens resemble those of NP, but the lens is also influenced by the sunlight.


76. The formation of ROS is initiated by an oxygen molecule absorbing an electron to form the superoxide ion \( O_2^- \), which may give rise to \( HO_2, H_2O_2 \), \( HO^- \) and further reactions such as peroxidation of lipids and proteins, and oxidizing cytosine in DNA to g 8-hydroxycytosin.\( HO^- \), a free radical with an un-paired electron, is most reactive.

79. Cross-linking in erythrocytmembran
81. Mitochondria – microtubule - dissociation
87. Yeast cell model of cancer stem cell
90. Spores from old yast cells
91. Contrary to cells of higher organisms, primitive cells are assumed to increase the amount of insoluble protein at cell division (c.f. the Lansing effect of yeast cells 89). It is interesting to speculate that this might have been a disadvantage early in evolution, avoided by the development of male and female gametes and meiotic divisions; fertilization by a spermatozoa means that the DNA content of the haploid oocyte is doubled without the need of protein synthesis, including the insoluble fraction.
92. Tomasetti C, Vogelstein B. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 347 (2015) 78-81.
94. Hall et al. Lipofuscin cause of aging
96. Caloric restriction