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A MECHANICAL SYSTEM INTERPRETATION OF THE NONLINEAR KINETICS OBSERVED IN BIOLOGICAL AGEING

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Abstract

There is a widely observed nonlinear kinetics in the ageing of biological systems which is characterized by three successive stages: (1) the ageing rate is firstly high but decreases quickly to a minimum from which (2) it remains nearly constant during the major part of the process until (3) it starts increasing again up to the final collapse of the system. Such kinetics are also encountered in the ageing of mechanical systems. It is shown that a model useful for the follow-up of operating mechanical systems allows to find back typical curves and laws related to the ageing of biological systems (mortality rate curves, survival curves, growth curves, Gompertz law, ...). The classical concepts of lifespan, longevity and life expectancy are given new light using the model, which also gives clues to explain both the discrepancy in the age of death of individuals in a given population and the wide range of lifespans of species encountered in nature. Finally, the model shows in which directions tests should be performed in order to accelerate te senescence for a better understanding of the underlying phenomena and for life prediction purposes.

Keywords

Ageing - Model - Biological systems - Feedback loops - Quantitative analysis - Lifetime

1. Introduction

Ageing in biology has been studied for decades both in theory and experiments. Many theories of ageing have been put forward. Some are based on individuals, eg. the Strehler-Mildvan theory which relates ageing to a diminution of energy reserves [48]. Other theories bind ageing to natural selection. In Kirkwood's disposable soma theory, it is suggested that ageing is the result of a life-long accumulation of random damage in somatic cells and tissues due to an evolved limitation in the levels of key maintenance functions. This idea predicts a central role for cell maintenance and stress response mechanisms in regulating the duration of life [26-28]. Another theory, the population genetics theory of ageing, postulates that as soon as the «reproduction» and «care of offsprings» functions have been completed, the individual is no longer useful under an evolutionary point of view [5]. Therefore, natural selection has tolerated defect accumulation and ageing.

Such theories do not explain why neighbouring individuals age with different kinetics. Also, the Strehler-Mildvan theory concentrates on a restricted period of ageing when physiological functions decline, not on the initial phase of growth and development.

In parallel to theoretical explanations as given above, a lot of phenomenological theories have been put forward based on observations and laboratory experiments. They can be distributed according to two main directions: (1) those referring to an accumulation of defects (the «wear and tear hypothesis») ; (2) those involving a genetic clock (the «programmed lifespan hypothesis»).

To the first direction, one can relate several studies which have put into evidence the functional changes connected to ageing. For instance, it is well known that the resistance to several factors (ultraviolet radiation, thermal shocks, etc.) and different functions - proliferative response of T lymphocytes to antigen [33], DNA repair capabilities [36,59], telomeric DNA [3, 55], volume of RNA transcription [17], etc. - diminish with age [41]. Also, the mitochondrial theory of ageing [12] is related to the kinetics of accumulation of defective mitochondria within the cells. This theory was modelled by Kowald and

Kirkwood [32] who also developed several theoretical models towards a network theory of ageing combining several intracellular « wear and tear » mechanisms [29-31].

Following the second direction of a genetic clock, we cite the often referred to «ageing clock genes» which would control senescence and death. Many authors support a genetically programmed lifespan limit because several *in vitro* studies have shown that cells have a limited capability of division [19,50]. Other studies have put into evidence the involvement of genes in ageing. For instance, it has been shown that a mutation in gene *daf-2* of the nematode worm *Caenorhabditis Elegans* more than doubles its adult lifespan on the condition that gene *daf-16* is active [25]. Similarly, a team managed to induce cellular senescence in immortalized hamster cells just by introducing human *chromosome 1* [49].

In 1986, after a rather extensive critical survey of theoretical models and quantitative observations related to ageing, Gavrilov and Gavrilova [16] summarize own work performed over a decade and discuss several models based on reliability theory. They conclude that there is no model which significantly outstands the others. Beginning of the nineties, Toussaint et al. unify several ageing theories within the paradigm of « critical threshold of error accumulation » [51].

The above mentioned theories and experiments attempt to track down the mechanisms underlying the process of ageing. However they strangely overlook a very commonly observed fact: that ageing in the broad sense develops in time according to a three-stage nonlinear kinetics.

In the present study, we show that this three-stage kinetics can easily be deduced from a systems analysis model used to assess the ageing of mechanical operating systems.

2. Evidence of the three-stage kinetics

The above cited studies are important because they provide tools to monitor the ageing process. One aspect is usually overlooked however: the fact that there is a first phase of growth and development which should be considered full part of the ageing process : eg.

for humans, the capabilities of immune response, of muscular resistance, of metabolic maintenance, etc. develop in a first phase during youth. Many authors separate growth and development from ageing. When however the word «ageing» is taken in the broad sense for a living system, ie. as the whole process of going from birth to death, strong nonlinearities appear. One usually observes three stages: (1) a first stage where the capabilities of the system increase : this is the « growth and development phase »; (2) a second stage where the biological functions stay at or close to their useful level: the «steady phase»; (3) a third stage of gradual decline of the functions, the «wear out phase». These three stages are widely encountered in nature as reflecting successive periods in the life of biological systems. Four types of curves are mainly observed : (1) «hill-shaped » , (2) survival, (3) mortality and (4) growth curves all of which show the three stages.

- "Hill-shaped" curves

For example, the cumulative increase in human diploid fibroblast cells number shows three stages [40]: the growth rate first increases, then stabilizes and finally gradually decreases. Three stages are also found for the dehydroepiandrosterone sulfate (DHEAS) concentration in human serum which, from the pioneer work of Baulieu [2], has been considered a good marker for the ageing of humans [42,47].

After a first increase, the DHEAS content in plasma reaches a peak around an age of twenty then continuously decreases. Similar curves are found when monitoring the quantitative changes in «tracers» accompanying the ageing of biological systems, eg: (1) the proliferative capacity of mouse immunocompetent T-cells [21], (2) maximal oxygen uptake in healthy fit men [61]; (3) incidental memory scores [60], etc.

- Survival curves

The three-stage nonlinearities are also found in a second kind of curves, the « survival » curves. The example shown in Fig. 1 corresponds to United States whites in the 1929-1931 period [7]. Similar curves can be found for other biological entities, eg *nematodes* [54]. One observes a drop in short age survival (corresponding to «infantile deaths»), followed by a period where the number of survivors is slowing down much more slowly.

This period ends with a new drop corresponding to the lifespan of the group of individuals considered

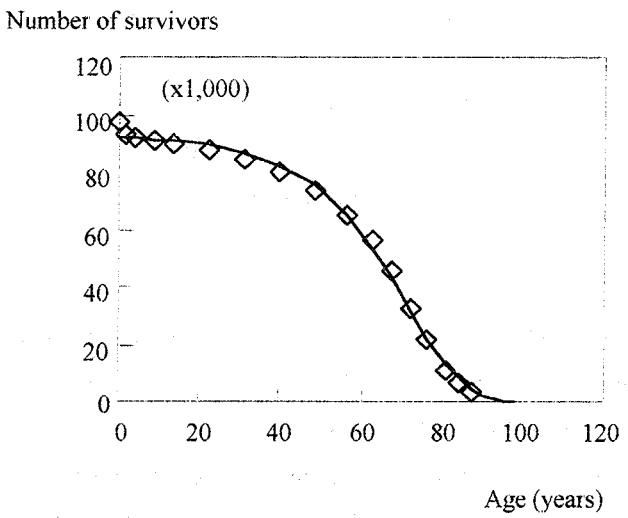

Figure 1. Survival curve of whites in the USA (period : 1929-1931).
As recorded (Comfort, ref. 7) (rhombs for visualizing)
As calculated (line) with the 3-stage kinetics model using Eq. (11) combined with Eq. (7) (C=92,283.34 ; $\alpha = 0.067 \text{ yrs}^{-1}$; $\beta = 0.25$; $k = 0.00292$).

- Mortality curves

Representations of a third kind of three-stage nonlinearities include « mortality » curves. Such curves give the mortality rate at different ages (or age groups) (e.g., Luder [39] or Shao, Gao, Yao, Zhuo and Riggs [45]). These curves show the three stages in the sequence: decreasing/minimum/increasing mortality. They are also found when analyzing the ageing of systems in *mammals*, eg. the immune system. Here, the populations are, for instance, lymphocytes. For instance, research on AIDS has also put the above three stages into evidence. There appears to be no latent phase after the first reaction to the virus. On the contrary, after the onset of the anti viral immune response, a steady-state is settled

reflecting a balance between virus multiplication and destruction. The *CD4+* lymphocyte depletion observed during the clinical latency is primarily a consequence of the destruction of these cells induced by *HIV-1* infection not a lack of their production while the virus is dormant [20]. Therefore, three phases are observed during the development of AIDS [6,57]: (1) reaction to the intruding virus ; (2) phase of steady resistance ; (3) exhaustion of the immune reaction in case of persistent action of the virus. This last phase appears when the virus cannot be definitely destroyed as a consequence of the fact that the regenerative capacity of the immune system is not infinite [37].

- Growth curves

Growth curves are found when the number of odd organic cells increases, e.g., in the growth of tumors in *mice* [15], in the increase of glial cells' number in the *CF1 mouse* cerebellum [4], in the increase of the percentage of multinuclear *chick* embryo cells in culture [24], etc. Such an increase of odd cells number is in agreement with the «wear and tear hypothesis» of ageing. As one expects the number of odd cells to be initially close to nil, a simple exponential extrapolation of measured data to time zero will usually be inaccurate. But then one must admit a curvature change from convex to concave (from below) towards zero as shown in Fig. 2. This is equivalent to admit a three-stage growth kinetics: (1) decreasing (2) steady and (3) increasing growth rate (slope of the tangent line).

One gets similar curves when plotting odd substances' accumulations in- and outside cells during the ageing process («wear and tear hypothesis» again). See, for instance, the intracellular accumulation of *human* peripheral nerve myelin by monocyte/macrophages with age [56].

The three-stage kinetics was already observed by Selye, the Hungarian/Canadian pioneer in the field of stress. He saw life as a three-stage adaptation to external stress agents called «stressors» [44]. According to him, ageing would be the extrapolation to life of his General Adaptation Syndrome (GAS). Let us recall that the GAS is the non specific internal reaction of the biological system to external attack and that it involves three

successive stages (underlining by the author): « [the general adaptation] syndrome is [an] expression of general defense divided into *three stages*. During the first, or acute stage, observed in the rat ordinarily 6 to 48 hours after the initial injury, one notes a *rapid decrease* in the size of the thymus, spleen, lymph glands and liver. After a few days, however, a *certain resistance is built up against the damaging stimulus*... the animals became resistant. If [the stressors] were continued still longer the *animals lost their resistance*, and in a *third stage died with organ changes similar to those seen in the first stage*. We have termed [the] 3 stages: the *stage of alarm*, the *stage of resistance* and the *stage of exhaustion*. » [43].

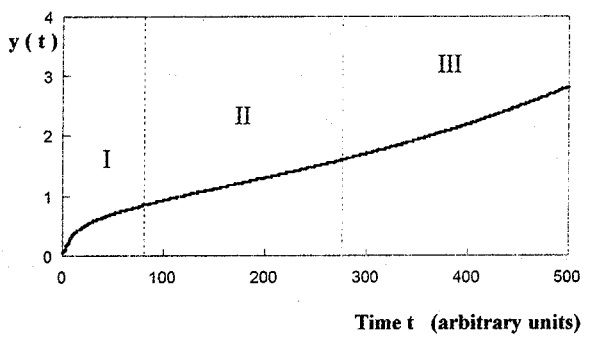

Fig. 2 : Global "ageing" curve.
This curve shows the evolution of a parameter descriptive of ageing with time. It results from using Eq. (4) & (5) for the example given in the text. E(t) as given by Eq. (7) will have the same shape.

3. Mechanical systems ageing

A minimum acceptable definition of a system is that it is a « group of interlinked subelements operating together with a common goal» [14]. Of course, this is an « afterthought » definition as the subelements did not necessarily decide to operate together in order to reach the goal. The links built up between the subelements may have been induced by the surrounding, but not necessarily with a specific goal (as is the case for

an engine built to drive a machine). Anyway, if the system works as an entity, it is thanks to the links between its subelements. The fact is there - it would not work anymore if it turned back to a situation where all links do not exist yet.

Therefore, in order to be more operational, we shall replace the words « with a common goal » of the above definition by the requirement that the system must have reached a state of «self-organized criticality». Self-organized criticality is a concept put forward by Bak, Tang & Wiesenfeld [1] on basis of observations on sandpiles and computer simulation of cellular automata. According to this concept, many composite systems (composed of many subelements) naturally evolve towards a critical state characterized by four facts:

- minor events can be at the origin of chain reactions which can affect any number of elements in the system: therefore, avalanches of events of any size can be produced ;
- the sizes of the avalanches are statistically distributed according to a power law with a negative exponent of the order of 1.2 ;
- this is true in space as well as in time;
- when the system is constrained to produce events, it tends, thanks to the avalanches, to return to a steady state of criticality (therefore, the expression «self-organized criticality»)

These facts specify what the words «group of subelements» mean: the elements are interlinked in such a way that when something happens to an element, this can have an influence on any other element.

Such systems are sometimes also called «complex systems».

Here, we define a system as: «**a group of subelements operating together and interlinked in such a way that the group shows self-organized criticality**».

Operation in a given environment necessarily introduces a time component and hence ageing kinetics of the system.

For mechanical systems, such kinetics can be modelled by the combination of many positive and negative first order feedback loops which occur for the subelements when

time elapses as a result of the step-by-step adaptation of the system to its operating conditions [9,10].

A very simple way to understand what a negative first order feedback loop means is the following. The operating of the system results in internal challenges on the subelements. The subelements have to adapt to the challenges in due time in order to allow further operation of the system as a whole. At each time, each subelement will modulate its available operating resources in a way to give the most appropriate response *y(t)* to the particular challenge *K* it has to meet.

The mathematical expression for a negative feedback loop is given by:

$$y(t) = K\,(1 - e^{-b\,t}) \qquad (1)$$

where: b : reverse of time constant for the response.

Let us now explain what a positive feedback loop is. During operation, the challenges are often not removed: they continue to appear sometimes on a steady base sometimes not, with given kinetics depending on the operating conditions. It may happen that because of the kinetics, the adaptations in the subelements are not perfect even if satisfactory for the further operation of the system. This may result in losses of information for neighbouring subelements about the challenges to meet in due time. Their responses will then become less perfect. There will be a snowball effect: imperfect responses will induce imperfect assessments of the challenges which in turn will induce other imperfect responses.

In a system, such behaviour corresponds to positive first order feedback loops. The relevant mathematical expression is an increasing exponential:

$$z(t) = z_0\,e^{a\,t} \qquad (2)$$

The macroscopic ageing kinetics of an operating mechanical system can be modelled as the result of the combination of multiple negative (corresponding to local adaptive responses) and positive (corresponding to local imperfect assessments of challenges)

feedback loops existing at subelement level at each time. We could simply express this as: «The ageing of a system will be reflected by an envelope curve resulting from the adaptations and dysfunctionings at sublevels»

Now, another concept appears in the literature about systems reliability : that of reliability growth. It corresponds to the observed fact that, for many operating inert systems, the failure rate firstly decreases with time towards a minimum value. In the case of mechanical systems, Duane called this the «learning curve» [13]. This behaviour is also referred to as corresponding to the «infant illnesses» of the system. Reliability growth results from the fact that the system is in a state of «self-organized criticality» which will be reflected by a statistical decrease of «*b*» in Eq. (1). The combination of multiple negative and positive first order feedback loops taking account of reliability growth will lead to global ageing curves of the kind shown in Fig. 2.

Let us illustrate this approach with a simple example. Take time increments of 10 units of time (*u.t.*). Suppose, in order to take account of reliability growth, that the «b_i» corresponding to the multiple negative feedback loops decrease as follows :

$$b_i(t) = \frac{1}{{t_i}^u} + v \qquad (3)$$

for: $t_{i-1} < t \le t_i \, (i = 1 \ldots n)$

If, for example, $u = 1.5$ (this is about the middle of the range often encountered for the inverse power law in self-organized criticality), $v = 0.01$, «*a*» in Eq. (2) is such that the timescale of the process is 400 *u.t.* ($a = 0.0025$) and $z_0 = 1$, we can write:

From $t = t_0$ to $t = t_1$ $\left(t_1 = t_0 + 10u.t\right)$,

$z(t) = 1$ and $y(t) = z(t)\left(1 - e^{-b_1(t)\,t}\right)$ (4)

From $t = t_{i-1}$ to $t = t_i$ $\left(t_i = t_{i-1} + 10u.t\right)$,

$$z(t) = e^{a\,t_{i-1}} \quad \text{and} \quad y(t) = y(t_{i-1}) + \left(z(t) - y(t_{i-1})\right)\left(1 - e^{-b_i\,(t-t_{i-1})}\right) \quad (5)$$

The evolution of $y(t)$ with time is shown in Fig. 2. We will call it the « ageing curve ». This example is one possible kind of combination of negative and positive feedback loops. It was chosen for sake of simplicity of the example. Other combinations can be made and/or other values of u, v, a and z_0 chosen. In order to get a global curve of the kind shown on Fig. 2, it is sufficient that :

- self-organized criticality be taken into account
- «a» in Eq. (2) be not that high that it masks the effect of the negative feedback loops.

What is usually found in the literature on the reliability of systems is not the curve of Fig. 2 but rather its derivative, which is called the «bathtub curve» because of its shape. The bathtub curve usually gives the failure rate for an operating system or for a class of systems of the same kind in function of time. A typical bathtub curve is shown in Fig. 3. Looking at this curve, we observe the three following stages: (1) a first stage where the failure rate quickly decreases with time; (2) a second stage where a stabilization occurs around a constant or slightly increasing failure rate ; (3) a third stage with an accelerating increase of the failure rate ending with rupture or wear out of the system: on Fig. 3, this stage has been divided into two parts: (1) before and (2) after the instability time (see later).

Such a behaviour was synthesized by following differential equation [9,10] derived from work by Duane [13], Cox & Lewis [8] and Lee [34]:

$$\frac{\dot{E}(t)}{E(t)} = \alpha + \frac{\beta}{t} \quad (6)$$

where: $E(t)$: measurable parameter which reflects the ageing process

α, β : constants ($0 \leq \alpha, \beta \leq 1$)

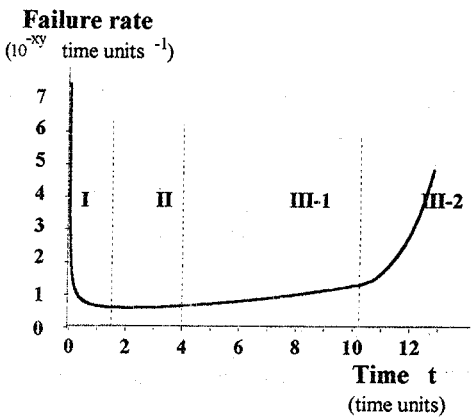

Fig. 3 : Typical "bathtub curve".

(This curve is called that way because its shape is alike ancient bathtubs. In systems reliability, it gives the rate of failures of a repairable system or of a group of identical systems with time)

It has again to be emphasized that, in an interpretation with multiple negative and positive feedback loops in subelements, Eq. (6) reflects the global ageing of the system as a whole. The feedback loops occur at a level which is several orders of magnitude (say $10^6 or 10^{10}$ or more, Avogadro's number - $6.023 10^{23} mol^{-1}$ - is an example of difference in order of magnitude) smaller than the level at which the system as a whole behaves. For a class of systems, the values of α, β may slightly change in function of the internal combination of feedback loops peculiar to each system. However, the key point is that the global ageing will always be described by Eq. (6) and the ageing rate have the shape of a bathtub curve (or part of it).

Eq. (6) appears to give a fairly general description of ageing. Once integrated, it gives - taking E(0)=0 - E(t) as a combination of an exponential and a power law:

$$E(t) = k\, e^{\alpha t}\, t^{\beta} \qquad (7)$$

When $0 < \alpha, \beta < 1$ and $\alpha << \beta$, we have a curve of the shape given in Fig. 2. This curve has been shown to reflect the ageing of several operating inert systems of different kinds: material, mechanical, electrical, chemical [9,10]

4. Meaning of parameters α and β

Let us now analyse the meaning of parameters α and β in Eq. (7). The parameter α stands for a pure exponential ageing pattern : it refers to the positive feedback loops ($\beta = 0; \dot{E} = \alpha\, E$: the ageing rate is at any time proportional to the level of ageing reached at that time). The reverse of α ($1/\alpha$) gives the order of magnitude of the timescale for the ageing process.

However, as already mentioned, a parameter β will appear in addition to α because of the adaptive behaviour to the operating conditions. This parameter will then rather refer to the negative feedback loops (α=0; $\dot{E} = \beta\, E/t$: the ageing rate will vanish at $t = \infty$)

Typical values of β will range between 0.15 and 0.65 for many systems [46]. In the example of Fig. 2, we find: $\beta = 0.421, \alpha = 0.00252(u.t)^{-1}$.

In fact, $1/\alpha$ physically corresponds to a time which we will call the «instability time t_i». It is indeed a general observation in physics that when the exponent of a time growing exponential reaches unity, unstable behaviour is to be expected. From the instability time t_i, there is a risk that the integrity of the system be lost. If the operating conditions are not relaxed, the system will eventually become impaired and behave irreversibly in an unstable way. The loss of integrity means loss of local adaptive responses in subelements inducing cumulative internal inadaptive behaviour up to general collapse of the system. Operation will usually continue after the instability time, but the process leading to the collapse of the system is irreversibly started. The best case to occur after t_i is that the internal inadaptations be distributed in such a way that they remain harmless and that the ageing of the system globally pursues the same path as before, up to exhaustion of all ressources for operation. The worst case appears when the start of instability induces a

quasi-instantaneous collapse. Both extremes will be very seldom. Most cases will be statistically distributed between the extremes depending on the kinetics of formation of one ore several macroscopic defect(s). Such macroscopic defects will result from the local focussed inadaptations. The period after t_i can thus be considered as a period of «unpredictable ageing». The ageing kinetics depends on the way inadaptations have locally focussed into defects within the system (these defects are seldom detected experimentally from the beginning with presently available techniques). This makes that the third stage must be divided into two parts: (1) before (predictable ageing) and (2) after (unpredictable or statistically predictable ageing) the instability time.

Therefore, we can distinguish several periods of time: (1) the instability time t_i; (2) the actual time of collapse of a given system t_r ; (3) the maximum possible lifetime for a class of systems t_m and (4) the statistical average of actual lifetimes of indivisuals in a class of systems t_μ.

Now let us further analyse Eq. (7). The three parameters α, β and k are constant as long as the physical constraints on the system remain constant. Let us explain this:

(1) The system has to operate under given conditions. In function of these conditions, the challenges on the subelements will vary in intensity. Severe operating conditions will induce challenges of higher intensity than milder ones.

(2) Moreover, the intensity of the challenges on each subelement will also depend on the internal organization of the system. A better organization will allow better adaptations at each time increment and lower the risk that non-adaptive responses be given to the challenges.

(3) Finally, physics command that ageing processes be temperature dependent (for example, Arrhenius plots should be observed).

Let us call these three constraints (1) the «stress Σ», (2) the «organization Ω» and (3) the «temperature T» respectively We thus have three more equations.

$$\alpha = \alpha(\Sigma, \Omega, T) \tag{8}$$

$$\beta = \beta(\Sigma, \Omega, T) \tag{9}$$

$$k = k(\Sigma, \Omega, T) \tag{10}$$

The particular form of these equations must be determined for each class of systems, for instance experimentally by fixing two constraints and letting the third one vary. Diagrams of the type schematized on Fig. 4, for example, can then be obtained depending on the relationships found between the parameters for a given class of systems with a given organization. Similar diagrams would be obtained with the time to end of life instead of the instability time.

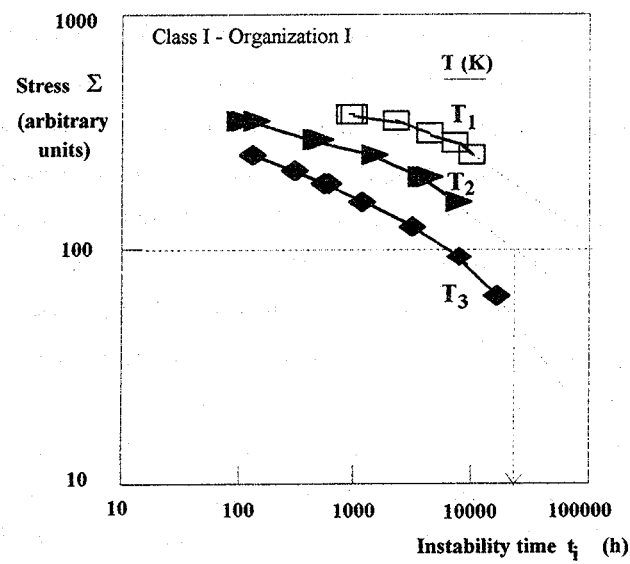

Fig. 4 : Schematic relationship between stress and instability time at different temperatures for a class of systems of given organization (squares, triangles and rhombs corresponding to measurements)

5. Biological systems ageing

According to the above definition of systems, biological entities as different as bacteria, cell, mammal, immune system, etc., all are systems. «Operation» for, e.g., the system mammal would mean «to live under given surrounding external conditions» (and : to maintain life, to favour the reproduction level of the species, etc.). Similarly, the purpose of the immune system will be to conserve immunity against external agents.

Although no systematic study has been performed yet on the subject, there are indications that self-organized criticality also exist in biological systems. For instance, it is well known that in some genes, one single mutation can result in several organic changes and even modify the phenotype. This corresponds to the first cited characteristic of SOC quoted above. Also main properties of all living entities such as homeostasis can be related to the fourth characteristic of SOC.

Going forward with the comparison to operating mechanical systems, negative first order feedback loops are also encountered in biological systems. For instance, homeothermy is typical of such loops. The system reacts to higher or lower external temperatures by metabolic adaptations allowing it to hold its internal temperature within a small range. Parameter b in Eq. (1) would then be the reverse of the time necessary to restore 63 % of homeothermy. As another example, it is known that some mutations in genes may increase the adequacy of the response to an external challenge, other not. Under steady challenge, natural selection will favour the most adapted individuals, ie. those for which gene mutations generate better response. Also, life prolonging mutations in genes, eg. in *nematode worms* [54], can be seen as mutations which increase the maintenance efficiency of these genes and thus the resistance of the worm to external factors. Lithgow and Kirkwood [38] therefore call these genes «stress-response genes», *ie.* genes that regulate the processes of maintenance and repair in order to overcome the effects of extrinsic stress. Such genes do therefore not necessarily regulate ageing as would a genetic ageing clock, but rather in such genes the defect formation is better counteracted thanks to the mutations. In a similar way, extended longevity is frequently associated with «enhanced metabolic capacity and response to stress» [22]. Again in all such cases,

parameter b in Eq (1) will be the reverse of the time necessary to restore 63 % of the property (maintenance efficiency, metabolic capacity, resistance to a weakening factor, ...) under external steady challenges.

Positive feedback loops are also found in biological systems. Many «snowball » effects fall into this category. Each time the rate of change of a parameter P at a given time is function of the level reached by the parameter at this time, Eq (2), ie. a growing exponential is obtained (this results from the integration of $dP/dt=P$). All cases of defects accumulation fall into this category. Mutations which are harmless in a first time may become deleterious when their effect is combined with other insignificant changes in the internal organization of a living entity, just because, for instance, there is a threshold in the number of these mutations and changes from which a metabolic function is impaired.

The idea that biological systems can - as inert systems - be modelled by the combination of many positive and negative first order feedback loops which occur for their subelements when time elapses as a result of step-by-step adaptations to the operating conditions, is in good agreement with several theories of ageing. For instance, in the disposable soma theory [26-28], the accumulation of random damage in somatic cells and tissues correspond to the positive feedback loops while the key maintenance functions correspond to the negative feedback loops. Also, it is recognized that adaptations to the environment (negative feedback loops) and some stochasticity both in the adaptations and in the damage production (positive feedback loops) are present in ageing. For instance, the limit of the number of divisions of cells in vitro [19, 50] is not necessarily a proof of the existence of a genetic clock. Indeed, even if genes do operate in the duration of the proliferative life, the control of the cells division is made together with their environment. Also, intracellular damaging factors lead to stochastic damage (as expected when there are many positive feedback loops) which need genetic repair processes modulated by the environment (negative feedback loops). And the instability time t_i probably corresponds to the critical threshold of error accumulation of Toussaint and co-workers [51].

Finally, the «learning » behaviour of biological systems is common evidence

We could summarize saying that the main difference between ageing inert and biological systems is that inert systems are put into operation by external agents (eg human technology) while biological systems are driven by the phenomenon of life. It is thus not astonishing that some authors describe life as the result of a succession of more and more elaborated inert systems put into operation by external agents [11]

Finally to consider an ageing biological entity as a system as defined above is in full agreement with Strehler's criteria of universality, progressiveness, eventual harmfulness and intrinsicality, to characterize a physiological process related to ageing:

- **Universality**: the negative and positive feedback loops are of the same kind for all members of an homogeneous group of biological entities, but may affect individuals to a different extent in function of their internal organization ;
- **Progressiveness**: the successive assessments and responses make that ageing is progressive with time (there is a kinetics at which changes occur);
- **Eventual harmfulness**: the accumulation of imperfect assessments makes that the process is eventually harmful to the biological entity;
- **Intrinsicality**: the ageing kinetics of a system result from internal adaptations to the operating conditions and environmental constraints which we could call « stressors ». These stressors induce internal « stresses » which may be defined in a general way as «distortions threatening the internal organization». The adaptations refer to the stresses not directly to the stressors. Identical stressors will induce different stresses, adaptations and ageing kinetics into individuals showing different internal organizations. This is in agreement with Strehler's criterion that any physiological phenomenon related to the ageing process must be intrinsic, ie age changes are restricted to those of endogenous origin (it is the internal response to the external stimulus which is age related, not the stimulus itself).

Therefore, we can try to extrapolate the model for the ageing of inert systems to biological systems. What would this mean ? What can we deduce that is useful for the understanding of biological systems ? Let us first analyse the biological meaning of parameters α, β and k in Eq. (7).

We have already noticed that the reverse of α ($1/\alpha$) gives the order of magnitude of the timescale for the ageing process. This will also be the case for biological ageing. For instance, the typical lifetime of humans will be 70-120 years ($\alpha \approx 2.6 - 4.5x10^{-10} s^{-1}$), while for a nematode worms, it will be 18 days ($\alpha \approx 5.7x10^{-7} s^{-1}$). However, as already mentioned, a parameter β will appear in addition to α because of the adaptive behaviour to the biological operating conditions. When there is no adaptation, the ageing will be purely exponential (β=0). The parameter k is a proportionality factor which gives the units for the measured phenomenon (value of the measure when α and $\beta = 0$).

We have seen that $1/\alpha$ also corresponds to the «instability time t_i». If the operating conditions are not relaxed after this time, the system will eventually become impaired and behave irreversibly in an unstable way. Alike inert systems, life of biological systems will usually continue after the instability time, but the process leading to the desagregation of the system is irreversibly started. Actual death will be normally distributed between two extremes : (1) t_i ; (2) the maximum time obtainable when all functions exhaust at the same pace (t_m). Death will usually depend on the kinetics of formation of one ore several macroscopic defects. Such macroscopic defects (eg. for *humans*: cancer, coronary thrombosis, etc.) will result from the locally focussed inadaptations.

The period after t_i can thus be considered a period of «unpredictable ageing». The ageing kinetics depends on the way inadaptations have locally focussed into defects within the system. And this cannot always be fully detected experimentally with presently available techniques. As a consequence, the third stage must be divided into two parts: (1) before (predictable ageing) and (2) after (unpredictable or statistically predictable ageing) the instability time t_i.

Therefore, we can distinguish several periods of time: (1) the instability time t_i ; (2) the actual time of collapse t_r (the «lifespan»); (3) the maximum possible lifetime for a class of living systems t_m (the «longevity») and (4) the statistical average of actual lifetimes of indivisuals in a class of living systems t_μ (the «life expectancy»). These times are visualized on the example of Fig. 5 (schematic with a timescale arbitrarily corresponding to *human* life).

The particular form of Eq. (8) to (10) should also be determined for each class of biological system, for instance in laboratory by fixing two constraints and letting the third one vary. Diagrams of the type schematized on Fig. 4 should then also be obtained for biological systems with the instability time or the time to end of life on the abscissa.

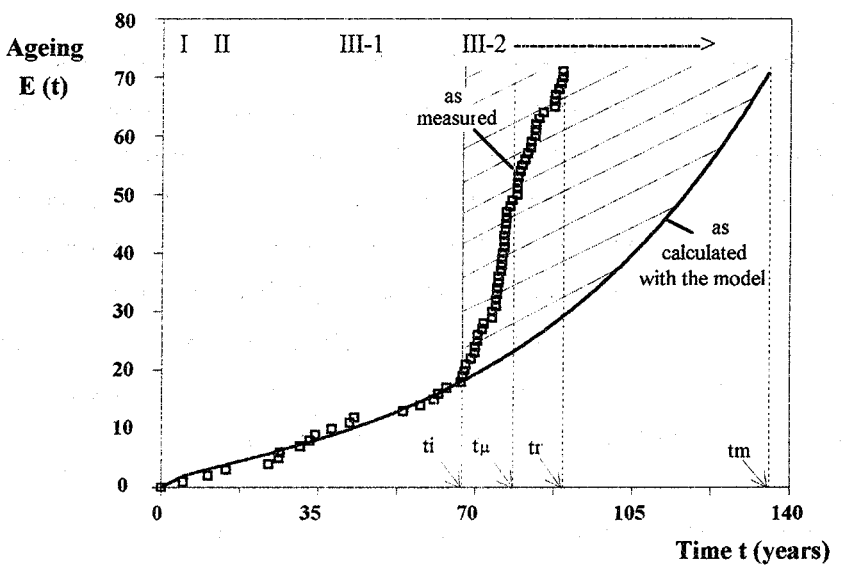

Fig. 5 : Comparison of measured and calculated ageing using the model of Eq. (7) (here the human life timescale was used as example) : ti is the instability time ; tμ the life expectancy, tr the lifespan for an individual and tm the longevity of the the species.

It is expected that under constant temperature (or stress) and same type of internal organization, both times - instability time and end-of-life time - will decrease with increasing stress (or temperature).

Indeed, k in Eq. (10) would logically have the following form for a given organization Ω:

$$k = \kappa \exp(-Q / R T) \Sigma^{b} \qquad (11)$$

(increase of ageing kinetics with temperature - following an Arrhenius-type relationship - and stress)

with : κ, b : constants (>0)

Q : reference energy of the process

R : gas constant

We thus obtain from Eq. (7) at t_i:

$$\ln t_i = \frac{1}{\beta}\left(\ln\frac{E_i}{\kappa} + \frac{Q}{R T}\right) - \frac{b}{\beta}\ln\Sigma \qquad (12)$$

with : $E_i = E(t_i)$

Eq. (12) gives diagrams of the kind of Fig. 4 (with $T_3 > T_2 > T_1$).

The above discussion is in full agreement with the approach of senescence adopted by Toussaint et al. [52,53] and should help devising physiological, cellular and molecular experiments. Of course, the increased stress must not be that high that sudden death occurs. For instance, accelerated cellular senescence should be produced by subcytotoxic stresses. Note that this is also common requirement in creep experiments on metals : the

stress level must be chosen to avoid instantaneous rupture such that there is a finite « creep lifetime » of the tested metal sample.

Finally, one could expect that both instability and end-of-life times will be the lower the worse the internal organization.

Establishing diagrams of the type of Fig. 4 through laboratory tests has thus the advantage to allow to quantify the influence of the stress, temperature and organization parameters on the senescence in the frame of the model given by Eq. (7) to (10).

It has another advantage : it allows to make quantitative predictions through (carefully tuned) extrapolations. This is shown schematically in Fig. 4 : under a stress Σ=100, the instability time will be around 30 000 h at T_2 and over 100 000 h at T_1. Such predictions can then be compared to observations.

6. Comparison with current curves and laws related to biological ageing

Now, if we take the reverse of the exponential of Eq. (7) (C: proportionality constant):

$$R = C.\exp(-E) \qquad (13)$$

we obtain the curve shown in Fig. 1 : this curve is usually called the «survival curve». Note that Eq. (13) is the classical «reliability » as used in the reliability analysis of systems.

Similarly, an « hill-shaped » curve can be obtained with an equation of the type $H = c.\exp(-\dot{E})$, but to our knowledge, there is no equivalent in systems reliability analysis.

Concerning classical methods of analyzing ageing processes in living systems, the two mathematical tools still considered the best are the Gompertz law and the Weibull distribution [23,35]. Gompertz law [18] gives a mortality rate proportional to $\alpha.exp(\alpha.t)$: combining Eq. (6) and (7) we obtain the same result if β is neglected, i.e., β=0. Indeed:

$$\dot{E} = E.(\alpha + \frac{\beta}{t}) = k.e^{\alpha t}.t^{\beta}.(\alpha + \frac{\beta}{t}) \qquad (14)$$

If $\beta = 0$:

$$\dot{E} = k.\alpha.e^{\alpha t} \qquad (15)$$

Therefore, in the present interpretation, Gompertz law results from neglecting the influence of the adaptation process (β=0).

Similarly, the 2-parameter Weibull distribution is given by [58]:

$$f(t) = \frac{\beta}{\gamma}.(\frac{t - t_0}{\gamma})^{\beta-1}.\exp\left[-(\frac{t - t_0}{\gamma})^{\beta}\right] \qquad (16)$$

$f(t)$: failure probability density

β, γ : parameters.

The corresponding hazard rate (or «conditional failure rate») is:

$$\lambda(t) = \frac{\beta}{\gamma}.(\frac{t - t_0}{\gamma})^{\beta-1} \qquad (17)$$

which, if $k = (\frac{1}{\gamma})^{\beta}$ and $t_0 = 0$, is identical to Eq. (14) with $\alpha = 0$.

7. Lifespan and longevity

One particular characteristic of the curve described by Eq. (7) is that it has an inflexion point at a given time t_2. This means that although the failure rate is low and nearly

constant during the second stage of the bathtub curve, there must be an absolute minimum at time t_2 (corresponding to the top level of operation). This time can easily be calculated and we find that $t_2 = (\sqrt{\beta} - \beta)/\alpha$. This can also be expressed by $t_2/t_i = \sqrt{\beta} - \beta$.

Calling « $E_2 = E(t_2)$ », « $E_i = E(t_i)$ », « $\dot{E}_2 = \dot{E}(t_2) = \sqrt{\beta}\left(E_2/t_2\right)$ » and « $\dot{E}_i = \dot{E}(t_i) = (1+\beta)\left(E_i/t_i\right)$ » the ageing level and ageing rate reached respectively at times t_2 and t_i, we find that t_i/t_2, E_i/E_2 and $\dot{E}_i/\dot{E}_2$ only depend on β. It is therefore possible, taking a range between 0,15-0,65 for β, to delimit an «instability zone» (corresponding to t_i) when t_2, E_2 or $\dot{E}_2$ are known. Fig. 6 shows the result. For instance, if $\beta = 0{,}5$ and $t_2 = 4$ years, the instability time t_i will be 19 years ($4{,}8 \times t_2$) at an ageing level about 5 times that at t_2. And the ageing rate will be about 2,1 times the rate at t_2.

Similarly, supposing t_2=17 years (eg. for a class of *humans*), the instability time t_i will lie between 68 (β=0.25) and 109 years (β=0.65). If then continuing the curve from t_i =68 years according to Eq. (7) gives a longevity t_m of 136 years, the lifespan t_r of an individual will lie somewhere between 68 and 136 years. Such figures would be expected in a population of *humans*.

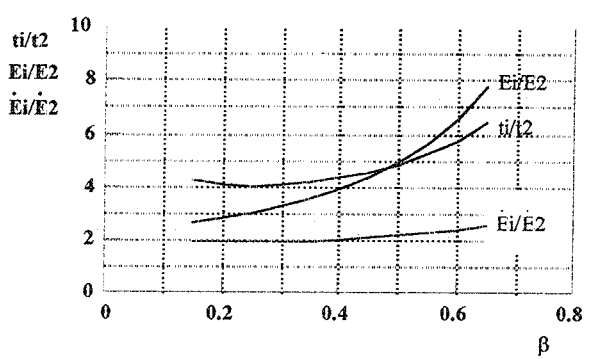

Fig. 6 : Start of "instability" as a function of β and values at t_2.

8. Conclusions

There is widely observed nonlinear kinetics for the ageing of biological entities which is characterized by three stages. In the present study, we suggest that, as in the ageing of operating mechanical systems, this results from the combination of multiple positive and negative first order feedback loops in the biological entity considered as a system. A mathematical model is proposed which allows to find back classical curves (eg. mortality and survival curves) and laws (eg. Gompertz law). It allows quantitative assessment of ageing and the prediction of the instability time from which the life of the entity might become irreversibly impaired. This type of a theoretical approach should help devising physiological, cellular and molecular experiments. It gives also the frame for the quantitative analysis of accelerated test results in order to assess the influence of parameters on senescence and to make useful extrapolations to the actual life conditions.

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References

[1] Bak, P., Tang, C. and Wiesenfeld, K., *Phys. Rev. Lett.*, 59 (1987) pp. 381 sq.

[2] Baulieu, E. E., *J. Clin. Endocrinol.*, *22* (1962) pp. 501.

[3] Bodnar, A. G., Ouellette, M., Frolkis, M. et al., *Science*, 279 (1998) pp. 349-352.

[4] Caron, P. C. and Unsworth, B. R., *Mech. Ageing Devel.*, 8 (1978) pp. 181-195.

[5] Charlesworth, B., *Evolution in Age-Structured Populations*, Cambridge University Press, 1980.

[6] Coffin, J. M., *Science*, 267 (1995) pp. 483-489.

[7] Comfort, A., *Ageing : The Biology of Senescence*, Elsevier, New York, 1979.

[8] Cox, D. R. & Lewis, P. A., *The Statistical Analysis of Series of Events*, Methuen, London, 1966.

[9] Crevecoeur, G. U., *IEEE Trans. on Rel.*, 42 (1) (1993) pp. 148-155.

[10] Crevecoeur, G. U., *Eur. J. of Mech. Eng.*, 39 (4) (1994) pp. 219-228.

[11] de Duve, Ch., *Vital Dust. Life as a Cosmic Imperative*, Basic Books, a division of HarperCollins Publishers, Inc., 1994.

[12] De Grey, A. D. N. J., *BioEssays*, 19 (1997) pp. 161-166.

[13] Duane, J. T., *IEEE Trans. Aerospace*, 2(2) (1964) pp. 563-566.

[14] Forrester, J. W., *Principles of systems*, Wright-Allen Press, 1968.

[15] Fotsis, T. & al., *Nature*, 368 (1994) pp. 237-239.

[16] Gavrilov, L. A. and Gavrilova, N. S., *The Biology of Life Span: A Quantitative Approach*, Harwood Academic Publishers, Chur (Switzerland), 1991 (initially published in Russian by: Nauka, Moscow, 1986).

[17] Gibas, M. A. and Harman, D., *J. Gerontol., 25* (1970) pp. 105-107.

[18] Gompertz, B., *Phil. Trans. R. Soc. Lond.*, A115 (1825) pp. 537-585.

[19] Hayflick, L. and Moorhead, P. S., *Exp. Cell Res., 25* (1961) pp. 585-621.

[20] Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. & Markowitz, M., *Nature*, 373 (1995) pp. 123-126.

[21] Hori, Y., Perkins, E. H. and Halsall, M. K., *Proc. Soc. Exp. Biol. Med., 144* (1973) pp. 48.

[22] Jazwinski, S. M., *Science*, 273 (1996) pp. 54-59.

[23] Juckett, D. A. & Rosenberg, B., *Mech. Ageing Devel.*, 69 (1993) pp. 1-31.

[24] Kaji, K. and Matsuo, M., *Mech. Ageing Devel.*, 8 (1978) pp. 233-239.

[25] Kenyon, C., Chang, J., Gensch, E., Rudner, A. and Tabtiang, R., *Nature, 366* (1993) pp. 461-464.

[26] Kirkwood, T. B. L., *Nature*, 270 (1977) pp. 301-304.

[27] Kirkwood, T. B. L. and Holliday, R., *Proc. R. Soc. Lond., Ser. B*, 205 (1979) pp 531-546

[28] Kirkwood, T. B. L. and Franceschi, C., *Ann. N. Y. Acad. Sci.*, 663 (1992) pp 412-417

[29] Kowald, A. and Kirkwood,T. B. L., *Mutat. Res.*, 295 (1993) pp. 93-103.

[30] Kowald, A. and Kirkwood, T. B. L., *J. Theor. Biol.*, 168 (1994) pp. 75-94.

[31] Kowald, A. and Kirkwood, T. B. L., *Mutat. Res.*, 316 (1996) pp. 209-236.

[32] Kowald, A. and Kirkwood, T. B. L., *J. Theor. Biol.*, 202 (2000) pp. 145-160.

[33] Kubbies, M., Schindler, D., Hoehn, H. and Robinovitch, P. S., *J. Cell. Physiol., 125* (1985) pp. 229.

[34] Lee, L., *Technometrics*, 22 (2) (1980) pp. 195-199.

[35] Lestienne, R., *Mech. Ageing and Devel.*, 42 (1988) pp. 197-214.

[36] Levitsky, E. L., *Gerontology*, 26 (1980) pp. 321-326.

[37] Levy, J. A., *Microbiological Reviews*, 57(1) (1993) pp. 183-289.

[38] Lithgow, G. J. & Kirkwood, T. B. L., *Science*, 273 (1996) p. 80.

[39] Luder, H. U., *Mech. Ageing and Devel.*, 67 (1993) pp. 247-259.

[40] Matsumura, T., Zerrudo, Z. and Hayflick, L., *J. Gerontol., 34* (1979) pp. 328-334.

[41] Medvedev, Z., *Mech. Ageing and Devel., 28* (1984) pp. 139-154.

[42] Orentreich, N., Brind, J. L., Rizer, R. L. and Vogelman, J. H. *J. Clin. Endocrinol. and Metab., 59(3)* (1984) pp. 551-554.

[43] Selye, H., *Endocrinology*, 21(2) (1937) pp. 169-188.

[44] Selye, H., *Stress Without Distress*, J.B.Lippincott Co., New York, 1974.

[45] Shao, Z., Gao, W., Yao, Y., Zhuo, Y. & Riggs, J. E., *Mech. Ageing and Devel.*, 67 (1993) pp. 239-246.

[46] Smith, D. J., *Reliability and Maintainability in Perspective*, MacMillan Education, Basingstoke, 1988.

[47] Smith, M. R. & al., *Clin. Chim. Acta, 65* (1975) pp. 5-13.

[48] Strehler, B. L. and Mildvan, A. S., *Science, 132* (1960) pp 14-21.

[49] Sugawara, O., Oshimura, M., Koi, M., Annab, L. A. and Barrett, J. C., *Science, 247* (1990) pp. 707-710.

[50] Swim, H. E., *Annual Review of Microbiology, 13* (1959) pp. 141-176.

[51] Toussaint, O., Raes, M. and Remacle, J., *Mech. Ageing and Devel.*, 61 (1991) pp. 45-64.

[52] Toussaint, O., Dumont, P. and Remacle, J., *Biochem. Soc. Trans., 24* (1996) p. 535S.

[53] Toussaint, O., Fuchs, S., Ronai, Z., Isoyama, N., Nitta, T., Petronilli, V., Bernardi, P., Gonos, S., Dumont, P. and Remacle, J., *Ann. N.-Y. Acad. Sci., 851* (1998) pp. 450-465.

[54] Van Voorhies, W. A., *Nature, 360* (1992) pp. 456-458.

[55] Vaziri, H., *Am. J. Genet., 52* (1993) pp. 661-667.

[56] Vlassara, H., *Age*, 11 (1988) pp. 74-78.

[57] Wei, X. & al., *Nature*, 373 (1995) pp. 117-122.

[58] Weibull, W., *J. of Applied Mechanics*, 51 (1951) pp. 293-297.

[59] Wheeler, K. T. and Lett, J. T., *Proc. Natl. Acad. Sci. U.S.A., 71* (1974) pp. 1862-1865.

[60] Willoughby, R. S., *J. Educ. Psychol., 20* (1929) pp. 671-682.

[61] Young, R. C., Borden, D. L. and Rachal, R. E., *Age, 10* (1987) pp. 138-145.

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