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DISCUSSION

Calculating Aging: Analysis of Survival Curves in the Norm and Pathology, Fluctuations in Mortality Dynamics, Characteristics of Lifespan Distribution, and Indicators of Lifespan Variation

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Abstract—The article describes the history of studies of survival data carried out at the Research Institute of Physico-Chemical Biology under the leadership of Academician V. P. Skulachev from 1970s until present, with special emphasis on the last decade. The use of accelerated failure time (AFT) model and analysis of coefficient of variation of lifespan (CV_{LS}) in addition to the Gompertz methods of analysis, allows to assess survival curves for the presence of temporal scaling (i.e., manifestation of accelerated aging), without changing the shape of survival curve with the same coefficient of variation. A modification of the AFT model that uses temporal scaling as the null hypothesis made it possible to distinguish between the quantitative and qualitative differences in the dynamics of aging. It was also shown that it is possible to compare the data on the survival of species characterized by the survival curves of the original shape (i.e., "flat" curves without a pronounced increase in the probability of death with age typical of slowly aging species), when considering the distribution of lifespan as a statistical random variable and comparing parameters of such distribution. Thus, it was demonstrated that the higher impact of mortality caused by external factors (background mortality) in addition to the age-dependent mortality, the higher the disorder of mortality values and the greater its difference from the calculated value characteristic of developed countries (15-20%). For comparison, CV_{LS} for the Paraguayan Ache Indians is 100% (57% if we exclude prepuberty individuals as suggested by Jones et al.). According to Skulachev, the next step is considering mortality fluctuations as a measure for the disorder of survival data. Visual evaluation of survival curves can already provide important data for subsequent analysis. Thus, Sokolov and Severin [1] found that mutations have different effects on the shape of survival curves. Type I survival curves generally retains their standard convex rectangular shape, while type II curves demonstrate a sharp increase in the mortality which makes them similar to a concave exponential curve with a stably high mortality rate. It is noteworthy that despite these differences, mutations in groups I and II are of a similar nature. They are associated (i) with "DNA metabolism" (DNA repair, transcription, and replication); (ii) protection against oxidative stress, associated with the activity of the transcription factor Nrf2, and (iii) regulation of proliferation, and (or these categories may overlap). However, these different mutations appear to produce the same result at the organismal level, namely, accelerated aging according to the Gompertz's law. This might be explained by the fact that all these mutations, each in its own unique way, either reduce the lifespan of cells or accelerate their transition to the senescent state, which supports the concept of Skulachev on the existence of multiple pathways of aging (chronic phenoptosis).

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Abbreviations: AFT, accelerated failure time model; CV, coefficient of variation; CV_{LS}, coefficient of variation of lifespan.

INTRODUCTION

This issue of *Biochemistry (Moscow)* is dedicated to the memory of the outstanding gerontologist and biochemist V. P. Skulachev. It includes the article [1] that mentions the very dialogue, in which it was suggested that not only the value of species-specific lifespan [2], but also its trajectory in a cohort (survival curve), as well as comparison of survival curves, are important for solving the problems in the studies of biology of lifespan, an area of gerontology developed at the Belozersky Research Institute of Physico-Chemical Biology.

The biology of lifespan has been the earliest research direction at the Belozersky Institute. It was initiated at the Department of Bioenergetics in the 1970s [3]. In 1991, a monograph was published in two languages, which has since become a classic textbook on this area of gerontology [4]. According to the Gompertz's law, an increase in the probability of death with age (at least, within a certain age interval) is described by the exponential dependence. Survival curves obey this law, at least in mammals and classical laboratory animals, such as nematodes and fruit flies (for more details, see [5, 6]).

As years passed by, it has become clear that not all problems could be solved by analyzing life tables using the Gompertz law (or Gompertz–Makeham equation) as a model for the increase in the risk of death with age, as it was revealed, for example, in the discussion about the limits of application of the Makeham term and derivatives of the Gompertz law (e.g., the Strehler–Mildvan correlation) [4-6]. Skulachev has become interested in the assumptions that are now discussed in the article [1]. Ten years ago, he created and headed a small group dedicated to studying these topics to which he contributed much of his attention (see [7-10]).

Sokolov and Severin [1] used the simplest method of preliminary research, namely, visual comparison of survival curves, in ten lines of mutant mice with progeria [11-17] and observed two types of survival curves. Type I curves were similar to those for control mice, while type II curves had (or resembled) an inverted exponential shape. Although it was only a preliminary analysis of survival data, it allowed to assess the severity of impact of the studied mutations on the survival curve of mutation carriers. In fact, normal survival curves are poorly approximated by an inverted exponential. This function [exp(-ct)] lacks inflection points, but has a long tail and is noticeably different from a straight line. In survival curves presented in [1], the inflection point is visible even on a step graph. This fact cannot be ignored when analyzing survival curves. After all, the presence of inflection point means the presence of long-livers, which are quite noticeable and cannot be explained by the error of measurements. For further data analysis, the Skulachev group used the methods discussed in the next section.

DATA ANALYSIS MODELS

The studies of Skulachev group. *Coefficient of variation (CV).* One of the main milestones in the research of lifespan was the article [18] published by Skulachev, Gavrilova, Gavrilov, and Severin (who is one of the authors of [1]). Using national population survey data from the United States and 14 most developed countries, the authors have moved from analyzing survival curves to studying the lifespan as a randomly distributed variable. They proposed that not only the lifespan itself and its time-dependent dynamics, but also its relative variation (e.g., CV) are important. It has been shown that the relative variabilities of the parameters of human development and aging are similar. Thus, the relative variabilities of age at which such an ontogenically controlled event as female puberty (menarche) occurs, age of the onset of aging-associated changes (menopause), and lifespan (age at death) are approximately the same, and their CVs fluctuate around 15-20% [18]. Later, analysis of data on the survival of Japanese women from an open database [19] produced similar results for the CV of lifespan (CV_{LS}) [7]. Indeed, all studies of additional demographic indices, including those suggested by Baudish, Vaupel, and Colchero [10, 20], began with the recognition of existence of methods capable of providing new information on the trajectories of mortality. Another significant step in the analysis of lifespan data was the work of researchers from the Max Planck Institute (Rostock, Germany/Odense, Denmark) published in 2014 in *Nature* [19]. Jones et al. [19] compared survival, mortality, and fertility curves for a wide range of systematic groups (23 vertebrate species, 10 invertebrates, 12 vascular plants, and one alga) from the age of sexual maturation to the age at which only 5% of individuals survived (terminal age).

Interestingly, Jones et al. in [19] failed to provide in the summary table the survival curves (and calculations of the studied parameters) for laboratory mice, despite the abundance of such data. The authors explain this by the fact that survival curves of mouse strains are greatly distorted compared to other animals (due to the load of mutations resulting from keeping these animals under laboratory conditions). Skulachev believed the work of Jones et al. to be a truly fundamental study that had summarized the most reliable data on the survival curves of a wide variety of species. He discussed the conclusions and ideas of this work at gerontological seminars and in his articles [10, 21] and even in his book "Life with No Aging" [22].

However, Skulachev suggested that despite the representativeness of the studied cohort, the conclusions of the authors of [19] were not that unambiguous and therefore, should be critically analyzed [7, 9]. Based on the calculations, the survival, mortality, and fertility curves published by the Institute of Demographic Research [19] were divided into four large groups according to the ratio between the mortality at the terminal age and average mortality within the entire studied period. Group I included species with the smallest increase in mortality with age (the ratio of the maximum mortality to the average mortality within the studied age interval), and group IV – with the greatest one [7]. Thus, in our opinion, the signs of actuarial aging are absent in plants and algae, some lower Metazoa (cnidarians), and some vertebrates (e.g., amphibians and reptiles). Beside mammals, species with a large number of postmitotic cells, for example, insects, are most susceptible to actuarial aging (although the mortality rate of other arthropods, such as crabs, may not increase with age). The limitations of the method [19] are most clearly seen in birds. Thus, contrary to the findings by Jones et al. [19], large birds age, but their biological aging might manifest itself at a late age to which less than 5% of individuals survive in nature. The aging of small birds, which have many enemies and high extrinsic mortality, is supposed to be almost impossible to detect in nature. Therefore, in the table, they would be located next to slowly aging species. The literature on biogerontology repeatedly mentions a situation when mortality caused by external causes is so high that it completely hides age-dependent component of mortality (a case similar to type II curves in [1]).

Semiparametric Cox proportional hazards model and accelerated failure time model (AFT). The survival curves of mice with mutations that shorten the lifespan differ (including the shape of the survival curve [pace and shape of aging]). They also look different in mice with progeric mutations, which not only reduce lifespan, but also lead to the development of age-related pathologies characteristic of this species. However, the influence of progeric mutations on the shape of survival curves (shapes of aging) is different in different cases. Since visual assessment of shapes of survival curves "by eye" does not always lead to accurate results (which is why at one time they abandoned eye tests, for example, the normality of distribution), special demographic methods began to be used in biogerontology. The semiparametric Cox proportional hazards model [23] is widely used in medicine and epidemiology, but rarely in the studies of aging. It estimates the rate of age-dependent mortality under different conditions by analyzing the log-cumulative hazard plot, i.e., dependence of the hazard ratio (logarithm of the hazard function) on the logarithm of time, which is useful in medical research (for example, for estimating mortality risks at various time points after surgery), but not for the analysis of survival curves [23, 24]. The AFT model compares entire survival curves instead of immediate probabilities of death at particular time points. In this case, survival curves can be transformed into each other by changing the variables: $S_1(\lambda t) = S_0(t)$, where λ is a dimensionless coefficient that determines the magnitude of the effect, which is the same for any quantile. The biological meaning of this formula is that biological clock runs at a different speed for the two compared groups of individuals. In this case, changes in the risk of death with age remain qualitatively the same. Graphically, survival curves S_1 and S_0 look stretched/compressed relative to each other along the time axis. At the same time, the values of the mean, median, and maximum lifespan also change proportionally, and the above-mentioned lifespan remains [almost] constant [25]. In this case, temporal scaling takes place, when various factors that either increase or decrease the lifespan (oxidative stress, changes in temperature or diet, mutations) do not change the shape of the survival curve, but only stretch or compress it along the time axis. According to the authors of [26], this indicates an existence of the aging program. In [25], raw data on the effects of various genetic changes that increase the lifespan in mice were analyzed using both models in order to select the model that most closely matched the experimental data. According to the AFT model, homozygous mutations *Prop1* ($\lambda = 1.48$) and *Pit1* ($\lambda = 1.39$) had the greatest effect on the mouse lifespan. The homozygous mutation *PappA* and heterozygous mutations *Clk1*$^{+/-}$ and *Irs2*$^{+/-}$ had a somewhat weaker effect in males ($1.20 < \lambda < 1.40$). The other genetic changes caused a similar and rather weak effect ($1.03 < \lambda < 1.20$). In the case of *Irs2*$^{+/-}$ mutation, the effect was stronger in males than in females, and in the case of *Clk1*$^{+/-}$, it was different in two mouse strains.

In 2016, an automated system was developed that allowed to accurately record the moment of death in *Caenorhabditis elegans* nematodes [26]. It has been shown that in nematodes, gene knockouts change the time-scaling coefficient of lifespan (λ) 2 to 3-fold, peroxide – up to 17-fold, and temperature – up to 7-fold [26]. Markov et al. [27] analyzed the survival curves of wild-type *Drosophila* flies, long-lived flies selected for slowed down aging (and, consequently, increased longevity), and short-lived flies that were cultivated on unfavorable food (selected for early reproduction). The authors assumed that with a small difference in the lifespan of the compared *Drosophila* groups, there would be a temporal scaling of survival curves (the Markov's rule). Skulachev and his colleagues [8] compared the data on the survival of *Drosophila* kept on normal and unfavorable (starch and salt, respectively) media [27] using the method proposed in [26]. Briefly, the lifespan data

were logarithmized and then normalized to a common time scale by dividing by the group mean lifespan. The mean value for the data transformed in this way was 1 in all groups. The deviations from the mean in different groups were compared in pairs using the Kolmogorov–Smirnov test with the Bonferroni correction [8] used for the multiple pairwise comparisons to reduce the likelihood of false-positive results [28]. The Markov's rule was shown to be valid for small differences in CV_{LS} (~10%) and non-overlapping survival curves and therefore, can be used for primary analysis of survival data [8].

THEORIES AND MECHANISMS OF AGING. KEY PROTEINS AND PATHWAYS REGULATING AGING

Aging is associated with degeneration of tissues in organs (e.g., atrophy of myofibrils in skeletal muscles and their replacement by cells of the adipose and connective tissues or an overall increase in the proportion of senescent cells). Based on this fact, the authors of [1] suggested that the primary cause of aging is aging of individual cells, and not, for example, age-related changes in intercellular structures. Sokolov and Severin [1] found that although mutations have different effects on the shape of survival curves, these mutations are of a similar nature. They are associated with (i) DNA metabolism (DNA repair, transcription, and replication); (ii) antioxidant protection related to the activity of the transcription factor Nrf2, and (iii) regulation of cell proliferation (although these categories can overlap as well). However, such diverse mutations appear to have a similar effect at the organismal level, namely, accelerated aging according to the Gompertz's law. This might be explained by the fact that such mutations, including mutations in the two genes coding for proteins responsible for the "true progerias" [Hutchinson–Gilford progeria ($Lmna^{G608G}$) and XPD (xeroderma pigmentosum D], each in their own unique way, either reduce cell lifespan or accelerate cell transition to the senescent state [1]. This supports the concept on the existence of the multiple aging pathways (chronic phenoptosis) proposed by Skulachev [10].

Considering the differences in the effect of mutations, we can assume that the effect of type II mutations on the lifespan is more detrimental. Once the probability of death ceases to depend on age (and lifespan decreases), then the viability decreases so much that animals die without having time to age more. This explains the fact cited in [1] that type II curves (with reduced lifespan) can be relatively easily changed. For example, deletion of the p21-encoding gene ($Cdkn^{-/-}$) increases the lifespan in mice deficient by the Terc1 telomerase, making the shape of the survival curve similar to an inverted exponential [29]. Transformation of a type I curve into a type II curve requires additional damaging effect and not a protective one.

Models of cell aging. According to [1], there are two types of experimental cell models of aging: replicative and chronological. Although both models have their limitations, they have been successfully used for testing geroprotectors [30]. However, A. N. Khokhlov [30], who was mentioned by the authors of [1], stipulated that the theory of replicative aging (and existence of the Hayflick limit) does not explain aging of an organism [31]. The statement that cell aging is the main cause of body aging is also a debatable issue. The authors of [1] refer to the Khokhlov's theory of aging. However, if we take his famous article in *Biogerontology* [32], it says something different: "Apparently, the impairment of regulatory processes, realized at the neurohumoral level, still plays the main role in the mechanisms of aging of multicellular organisms, not just the accumulation of macromolecular defects in individual cells. It seems that the quality of the cells themselves does not worsen with age as much as reliability of the organism control over cells, organs, and tissues, which leads to an increase in the probability of death". Similarly, Skulachev has repeatedly noted the importance of the control of aging at the body level, namely the action of biological master clock located in the cells of the suprachiasmatic nucleus of the hypothalamus.

Indeed, when studying the effect of mutations, it cannot be excluded that genes knockout will not affect other processes at the same time, therefore, such studies should be focused on individual genes or proteins that play an important role in a specific mechanism investigated in each specific study. Thus, for the Khokhlov's group, which studies DNA damage as a primary cause of aging, such protein is PARP1 (a universal sensor of DNA damage) [33]. Skulachev, in turn, considered the Nrf2/Keap1/ARE system as one of the most important representatives of the anti-aging programs (vitauct) [34]. This system controls almost entire cell antioxidant defense [35], in particular, expression of genes responsible for cell protection from the oxidative stress and mentioned in [1] (expression of such genes is under control of Nrf2 due to the presence of ARE in their promoters; also, p62 protein protects Nrf2 from proteasomal degradation with the participation of ubiquitin ligase adapter Keap1).

In conclusion, I would like to support Severin [1] in his suggestion to continue gerontological research in the directions outlined by the Skulachev's group. In particular, it would be very valuable to resume our weekly gerontological seminars for discussion of current research studies and news in the field of biogerontology. These seminars had attracted not only

employees of the Institute of Physico-Chemical Biology and the Faculty of Biology of the Lomonosov Moscow State University, but also many scientists from other institutes, which once again confirms the relevance of the topics discussed at these meetings.

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