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How to Cancel the Program of Body Aging?

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Abstract—This issue is devoted to biochemical aspects of studies on aging of living organisms. During last several years, new evidence was published to show that aging is programmed by the genome. If this is really the case, there is a chance to prevent aging just as the programmed cell death (apoptosis) can be prevented by inhibitors arresting some steps of this process. That is why the main problem discussed by the majority of the authors contributing to this issue consists in an attempt to answer the question whether aging is programmed or not. At present it is hardly possible to solve this problem. However, we already can assume that at least there are no facts allowing to exclude existence of the aging program in humans. Thus, there is a perspective to invent a chemical agent competent in transferring humans from aging to non-aging creatures, which is exemplified by the naked mole rat whose death rate is independent of age.

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I will never beleive that a horse and
a horse vehicle age in a similar way!
Alex Comfort

Definitions of Certain Terms

The articles constituting these issue represent different points of view on the debated subject. To compare them, we first of all should agree about what we mean by the term "aging." Here is one of the definitions of this term: "Aging is a slow and concordant decay of body functions with age." But for the word "concordant," such definition could pretend to commonly accepted. And while the concordant decay of separate functions is an obvious fact, most gerontologists will hardly agree with it. On the one hand, it is quite clear that in such an intricate system as an organism it is impossible to impair any one function not adjusting other its associated function in accordance with the worthened situation. Maldajustment would lead to a fast death without any aging. On the other hand, is not it waste of effort to adjust something, if things are at all always going to a sad final? Here we come to a dilemma: Whether aging plays any positive role in the evolution of live organisms or it is an inevitable result of their wearing?

To pass to consideration of this key issue of modern gerontology, we need to introduce one linguistic clarification. These are two terms in the English language: aging (or ageing) and senescence. By ag(e)ing, in a strict sense, is meant an increase of the age of an object and senescence, an age-related deterioration of qualities of an object due to its wearing. Possessing sophisticated reparation systems, a live organism should not necessarily wear with age. And indeed, there are species, including mammals, that live very long but do not undergo senescence as they are getting older and older. The reasons of their death are either age-unrelated and unknown or (in endless growing species) result from steadily enhancing disproportions in the sizes of separate organs (for example, shell and muscles that maintain muscles in a right position or moving it in space, vide infra). In any case, there is no a grain of evidence here for the decay with age of vital functions as a reason of death.

Aging as a Program

The term senescence has a disadvantage that it relates exclusively to the final stage of age-related decay of body functions, when the life way has already been passed, and such an important quality as the capacity for reproduction is sharly attenuated or lost at all. At the same time, certain vitally important functions start to decay very early, when all other

functions (incluing reproductive) are still in order. Certain immunity systems start to decay in a 15-year age [1]; wound repair and vision accomodation functions, too, start to decay at the same time [2]. Sarcopenia (reduction in skeletal muscle mass) developes soon after a 20-year age, visual acquity impairs in a 30-year age, lung volume reduces in a 35-year age, and skin elasticity decays at 45 [2]. Therefore, it is not surprising that the age-related exponential growth of death probability commences in a 15-year age [2].

It is not excluded that there are random reasons that different body functions start to decay in different ages. Thus, details of a certain mechanical device, made of metal, will wear slower than those made of wood. At the same time, it seems strange that sarcopenia in skeletal muscles develops quite early but is lacking in the cardiac muscle, even though the former work from time to time, whereas the latter works continuously throughout all our life.

An alternative explanation of the situation consists in that sarcopenia and other age-related functional decay processes are a manifestation of the implementation of a special program contained in the genome. This program is evidently couterproductive for an individual but may prove useful for the evolution of a species. Any new trait which gives a small advantage to its acquired individual may turn to be neglected by natural selection, if this relates to strong young individuals. The same trait obtains a chance to be selected by evolution, if it will turn to be vitally important for organisms weakened by aging [3,4]. Let us consider the following example. Two young hares: one more clever and the second not as clever, when met a fox, have almost equal chances to escape from the enemy, since they can run faster. However, with age, the clever hare will gain advantage over the stupid one, since they will run slower due to sarcopenia. Now the clever hare, as soon as he notices a fox, will immediately take to heels and has much more chances to save himself, than the stupid one which will be delayed at the start. This means that the clever hare will proceed with multiplying baby hares [21]. Essentially, the principal, most actively reproduced, young part of the population will not be involved in such experiment, being a guarantor of the stability of what has already been reached by evolution. At the same time, the aging part of the population can afford to slightly change the genotype of the species by selecting certain new properties. If these properties will prove actually useful, they will eventually be fixed. Therewith, the threat of unfavorable side effects destructive of the species is minimized, since the fraction of old individuals is not so large, they reproduce not so actively, and will simply die soon.

Examples of Biological Programs Couterproductive for Organism

Biologists know numerous examples of programs couterproductive for an organism but useful for evolution. They include, for example, suicides of male individuals after mating with female ones. Obviously, this act should favor a more diverse posterity: Since an individual can only once become a father, every next litter will comprise descendants of another father. In certain spider species, a male promts a much bigger female to kill it immediately after coitus. Ejaculation in a male mantis becomes possible only after his decapitation which, too, is a function of a female mantis during coitus [5]. A male squid sinks immediately after he has managed to deposite his spermatophore into a female genital tract [6]. Sometimes females are sacrificed. Thus, a female bumble bee two-spot octopus *Oktopus filosus* loses appetite and dies of starvation, when babies have hatched and she no longer needs to guard laying from marine predators. Removal from the brain of a female octopus of so-called optic glands switches off the suicide program, and this female can reproduce many times [7].

Pacific salmon before spawning transforms into humpbacked salmon, a ridiculous humpbacked creature with a mouse unsuitable for eating. Humpbacked salmon will die immediately after spawning. The death of salmon resembles a fastened movie in which the entire aging program is implemented over a couple of weeks, beginning with immunity decay and ending with osteoporosis in bones and amyloid placques in the brain. Zoologists suggested for a long time that the accelerated aging of salmon is a concequence of a heavy work it performs swimming to the origin of a river against its flow, and this way is sometimes a thousand kilometers long. This explanation failed, after two circumstances were made clear. First, fish with removed gonads or adrenals does not experience aging. Second, the transformation into humpbacked salmon is observed even when the spawning site is separated from ocean by a channel as short as several hundreds meters [8]. One of the possible explanations for the death of salmons after spawning consists in that

parents sacrifice themselves to give food for their babies: Humpbacked salmon bodies are eaten by maxillopoda which, in their turn, are eaten by salmon babies.

An interesting suicide case in Australian pocket mouse is described. Male animals die two-weeks after estrum, under the action of their own pheromones which they initially release to attract female animals [9]. It is striking that a similar mechanism exists even in yeast. Yeast cells release pheromones to stimulate sexual reproduction of partner cells of the opposite sex. In cases where mating has been delayed by a certain reason, excess pheromones released in the gap between the two stuck cells and kill these cells. By our data, the mechanism of this phenomenon is quite similar to apoptosis, i.e. programmed cell death in multicellar organisms [10]. The biological sense of this effect might consist in a stimulation of the transition of yeast colonies from vegetative to sexual reproduction as a response of single-celluar organisms to impaired external conditions. In essence, pheromones sort out cells which turned to be incapable of such a transition [11].

Self-destruction phenomena are frequent in plants. Thus, soy beans, when ripe, release some substances which kill the plant. If the beans are timely removed, soy can transform from an annual to a perennial plant under favorable conditions. Bamboo reproduces vegetatively for about 15–20 years, then it flowers, form seeds in a middle of summer, and dies. As a result, parents not only free space for their descendants, but also enrich soil for them [12]. The alternative explanation, namely, the plant dies because it is exhausted by forming seeds, is unlikely. In the last year botanists described a mass death of Madagascar's giant palms. The palms broke into bloom for the first time in a century and immediately died. Comparatively small inflorescences resembling asparagus in appearance and size were surely unable to exhaust giant trees which are clearly seen in satellite images.

Phenoptosis

The above examples all provide clear evidence showing that there is a tool in the live nature for preventing the natural selection from sorting out the programs couterproductive for inividuals but useful for the evolution of their genome. If the implementation of such programs leads to a death of the individuals, we suggest to define them by the term *phenoptosis* or *biochemical suicide of an organism* [4, 13, 14]. The above-described cases of suicides attendant in sexual reproduction or directly resulting from it can be classified as an *acute phenoptosis* which fast develops with time. As seen from the example with Pacific salmon, the acute phenoptosis is a sharply accelerated aging with all principal signs of senescence; this process takes years in humans and only days in salmon. This fact alone is a strong evidence showing that human aging is contolled by a program which differs from the acute phenoptosis in salmon by the time scale only. In this case, a usual aging could be defined by a *slow phenoptosis* or the biochemical suicide strongly streched in time.

Some interesting evidence in favor of this assumption is provided by the research on aging of a small African fish *Nothobranchius furzeri* which lives in puddles formed in the rainy season and dried after this period which lasts four months. It is not surprising that the sexual maturation of *N. furzeri* occurs very rapily, over the first two weeks of life. Then the fish mates many times and lays eggs up to the end of the fourth month. Wondeful is another circumstance: Over the short period determined for it by the habitat conditions, the fish *manages to age*, manifesting by the end of its life a whole set of signs of senescence, including amyloid placques in the brain [15]. This fact is unambiguous evidence to show that aging is a stage of individual development, and the organism daren't sacrifice it even in extremely unfavorable conditions. Clearly, for *N. furzeri* this is not the way to remove incapable individuals from the population of (such role was assigned to aging by Weismann [12]), since all inhabitants of the puddle are all the same doomed to death by the end of the four-month rainy season.

At present we cannot specify exactly all functions of aging. One of the possibilities was mentioned above (a way to "pull out from under noises" a new positive trait which is too weak to be noticed by natural selection in young and strong individuals). One more possibility is mentioned by Goldsmith in his review in this issue of the journal [16]. Were organisms not aged, with age they would acquire more and more advantages over young organisms, first, by being enriched with experience and, second, by increasing the assortment of antibodies to different diseases which they have already suffered. (The second circumstance makes clear why the immune system in animals start to age earlier that other body systems.) Here we face an ambiguity in the requirements for successful expansion of the genome of one or another

live species in the biosphere. On the one hand, it is necessary to conserve all what has already been acquired (*conservatism* of the genome) and on the other, to provide its *variability* and, consequently, progressive evolution. From the conservative point of view, young individuals which have not yet accumulated the mutational load increasing with age should not lose to older ones. An interesting idea consists in that cancer is a phenoptotic program which sorts out individuals with an excess mutational load (cf. the review of Manskikh [17]).

Arguments of Opponents of Programmed Aging

The key counterargument of the opponents of programmed aging, and they still form an overwhelming majority among gerontologists (see the review of Khokhlov [18]), is that the properties counterproductive for an individual are impossible to conserve in the evolution. Therewith, the opposite point of view is interpreted as anti-Darwinism. However, one can find in Darwin's "The Descent of Man" the following strings: " There is no doubt that a tribe comprising a great many of individuals which…. are always ready to support each other and sactifice themselves for the public weal will always defeat other tribes, and there will be a natural selection" [19].

Another counterargument consists in that the scatter in individual lifespans is too large to explain their death of senescence in terms of a program. However, it is appropriate to remember the first strings of the "Anna Karenina" novel: "All happy families are similar to each other, each unfortunate family is unfortunate in its own way" [20]. This is just the fact that not one but many vital functions decay with age, which suggests a variety of concrete reasons for the death of senescence, and, as a consequence, everybody has his allotted lifespan.

Objections are raised that aging is an artifact characteristic of laboratory animals, and wild animals don't make old bones. This is an obvious confusion, associated with a common misthought that aging and senescence are in essence one and the same phenomenon. We already mentioned above that aging commences much earlier than deadly body senescence occur. There is a great body of direct evidence for a correlation between the mortality of wild animals and their age in the wild (cf. [8]). This effect is contributed by a minor (but significant) decay of those vital functions which are the first to be responsible for switching on the aging program. The decay of the immune system leads to an age-related increase of the rate of deaths of infections, progressive sarcopenia enhances the probability to be killed by a predator, and sight weakening enhances the probability to face it. There and similar circumstances are commonly not considered "death of senescence," but they are surely directly related to a commencement of aging in still fairly young organisms.

One more counterargument: Why evolution devised aging as a tool of self-destruction, is an organism is so complex that it will all the same break down. First, self-destruction can be a final result but not a function of aging which is needed to drive evolution [3, 16], retain the conservatism of the genome (see above and Goldsmith's review [16]) or some other function useful for the genome from the evolutionary viewpoint but counterproductive for an individual. Second, we face here with a particular case of a "dictatorship" of the genome, when its interests dominate over the interests of an individual [5, 21] (for detail, see the last section of this review).

Unaging Beings

The opponents of the program concept frequently ask why there are no immortal mutants in which this program is spoiled? Comfort [2] reasonably notes [2] that switching off the aging program does not imply immortality or even a manyfold increase of lifespan. One can be able to live long even if the aging program does not work. Quite instructive are the observations on unaging species. Such species members die not of gradual decay of many physiological functions but of age-unrelated reasons (say, an accident) or of a single age-related change incompatible with further existence.

Thus a pearl clam which grows all over its life dies because the shell valves become too heavy for the muscle which keeps the organism vertical at the river bottom. This muscle also grows with age but not as fast as shell valves. Probably, a similar reason (disproportional growth of shell and leg muscles) explains the death of gigantic tortoises which, too, grow all over their life and finally lose mobility. Both the above-mentioned species live longer than two centuries showing no signs of senescence. Their only competitor in terms of lifespan is Greenland right whale. The reason of his death is unknown, but it was shown that at times of about two centuries the spontaneous L→D isomerization of amino acids in eye

lens crystalline proteins is above 20% [22], which may prove critical for the animal sight (as known, eye lens crystallines are not replaced all over the life, and, therefore, the isomerization of amino acids may destruct these proteins and lead to lenticular opacity).

Unaging beings include, along with pearl clam, big tortoises and whales, as well as gigantic crabs, huge Arctic perches, pikes, crocodiles, and certain big island birds. The common feature of the above beings is that they don't have enemies and, therefore, the pressure of natural selection on them is sharply weakened. Under these conditions, the aging program serving to this selection proves to be unnecessary. The loss of this program due to random mutations does not entail negative consequences and simultaneously improves survivability of individuals. Such mutations should be conserved by evolution. Having found an enemy-free niche, a species can afford longevity, even though it inevitably retards change of generations, and, a result, evolution, which gets not so essential in the enemy-free medium. With time, long-lived species better and better adapt to a long life, so that not to subject any age-related changes which may intefere with fulfilling a definite vital function. Thus, if Greenland right whale has to die of blindiness caused by the racemization of amino acids in lens crystalline, then its further evolution should be targeted at a replacement of old cryslallines by new ones, say, every hundred years of the animal life.

This is just the inadaptability to a long life, mutations in the aging program of usual species cannot radically (manyfold) prolong the lifespan of individuals, even though some reported precedents can be mentioned. A more detailed analysis of such cases leads to a conclusion that we deal here with a switch-off of one (short-lived) phenotype to another (long-lived) which, too, pre-exists in an individual but does not manifest itself in normal conditions. Thus, sensational results were obtained in the research on a mutant of the filamentous fungus *Podospora anserina*, when a point mutation in a gene encoding a subunit of the cytochrome *c* oxidase, inactivated this enzyme and prolonged the lifespan of the fungi more than 20 times [23, 24]. However, the authors of the cited works neglected the fact that the same fungi can live virtually indefinitely long, reproducing vegetatively in a suspension culture [25]. Apparently, this mutation switched off *Podospora anserina* to a different modus vivendi which it kept in store when cultivated in agar. Lakowski and Hekimi [26] are likely to face the same situation in their research on the nematode *Caenothabditis elegants* whose lifespan could, too, be manyfold prolonged by mutations [26]. The case in point is that in unfavorable conditions this worm tends to fall into an inhibited state (dauer) which features a low metabolism and a long life.

Naked Mole Rat

The very fact of existence of unaging beings even among the most complex higher organisms, such as mammals and birds, should be quite unpleasant for those who denies programmed aging. Actually, if a horse aged in the same way as a cart (all complex things tend to wear and break down with time), then the lack of aging would be expected in only the most primitive, simply organized living beings.

Until recently those who studied unaging higher organisms faced one great inconvenience associated with the lack of an experimental laboratory animal possessing this trait. Even if you are interested in unaging mammals, you won't experimentate on Greenland right whale. An invaluable gift to advocates of programmed aging was made a few years ago by the American researcher Rochelle Buffenstein who found that the mortality rate of an African rodent called naked mole rat (*Heterocephalus glaber*) does not increase with age. The wonderful features of naked mole rat are described in detail in the review of Mele et al. in the present issure of this journal [27].

H. glaber, a rodent of the size of a mouse, was discovered by the zoologist Ruppel in the Equatorial Africa. For his appearance resembling a mouse embryo ceased to develop, it was initially percieved as an underdeveloped freak from the rodent tribe. However, later some evidence was obtained showing that naked mole rat can run rings around any of its tribsmen. It was found that it "devised" a sophisticated social life arrangement whose rules are undeviatingly followed by all members of a colony comprising 200–300 individuals. Naked mole rats live in tunnel systems they build in rocky ground (1/3 of all muscles of this animal falls on the muscles of incisive teeth capable to gnaw through concrete). These tunnel systems are about two "football fields" in size. The center of the underground labyrinth is allotted to the queen's apartments and a common lavatory. The queen breeds for the whole colony. It has one to three breeding males which are, too, generally permanent (sometimes one of them is replaced by the most brave and strong male from a neighboring colony with which all the colony

members fight for deal life in all other respects). Mole rates also fight with snakes which sometimes creep into the labyrinth to make a feast of rodents. However, warfare is the share of common colony members who guards the queen and breeding males. The queen of naked mole rats, unlike bee and ant queens, are genetically identical to any of its homagers.

Naked mole rats eat plant tubers, digesting them completely (including fiber), for which they have special bacteria in the intestine. They live up to 28 years (cf. mice whose age limit is usually 2.5–3 years). Over the course of her 30-year-long experiment on 500 naked mole rats, Buffenstein never could establish with sure the reason of their death. These animals don't suffer cancer, cardiovascular diseases, or diabetes and exhibit a high immunity to infections over the whole life. In captivity they cannot live alone. They are maintained in small groups in which the post of queen is abolished, all animals breed and live long, and, therewith, the probability to die in old individuals is as low as in young. In the wild the queen is extremely hostile to other females (which are few in number and comprise about one third of the male population) and does not let them to breed. Furthermore, in the wild the priviledge of longevity is realized exclusively by monarches, whereas workers die not reaching a 3-year-old age, falling victims to wars with snakes and workers from other naked-mole-rat communities. As seen, workers have fierce enemies, unlike the queen and its husbands, who are reliably protected from them by the whole army of nonbreeding inividuals. Since only breeding individuals can contribute to evolution, naked mole rat should be classed with unaging long-livers. It is interesting that this species, too, fits the above formulated rule: Unaging individuals contributing in the evolution process do not have enemies.

It can be suggested that naked mole rats devised a modus vivendi in which the functions of getting food and defending from enemies, on the one hand, and the fuctions of self-reproduction (and, consequently, participation in evolution), on the other, are allotted to different representatives of the community. These two cohorts (working but not breeding and breeding but not working) are genetically identical, and in the wild they form anew every time when the animals establish a new colony. This colony should have a sufficient number of members to be able to provide a reliable protection of the new queen. In laboratory conditions, when the size of the colony is limited to a few individuals, no queen is selected, and the indiviuals all are involved in breeding.

It is striking how perfectly the breeding female of naked mole rat is adapted to the reprouction function. It gives birth to an unusually great number of pups (up to 30 in one litter). Gestation lasts 11 weeks. Over this term the female body weight doubles, and, therewith, the animal size increases due to body lengthening. The backbone lengthens (a unique trait developed as an adaptation to the underground existence). The metabolism accelerates 3 times. Very soon after delivery the queen is impregnated again, and this cycle repeats all over her long life. Naked mole rats lack such a characteristic feature of old females as a cessation of regular estrous cycles. The most probable rationale of the naked-mole-rat phenomenon consists in that these animals lost the aging program, since it proved to be unnecessary due to the comfortable conditions for breeding colony members which do not have enemies and, as a result, no longer experience the pressure of natural selection [21].

Why Gerontologists Do Not Like the Idea of Programmed Aging?

In one of the previous sections we specially dwelt on the arguments of opponents of programmed aging to show that, in essence, no one of these arguments does not stand up. What is the explanation for the fact that a great number of gerontologists dismiss any thought about the existence of such program already for over more than a century? The primary reason for the persistence and common acceptance of this, in our opinion, misbeleif is that most biologists do not undestand who is the host in the "biological home." They are still completing the Darwin's law on "the evolution as a victory by means of natural selection of the most adapted," by adding to it only one word: "individuals." We already cited above the Darwin's book about the descent of man, where he admits altruism or even self-sacrifice for the welfare of the tribe. We also considered the case of naked mole rat, when 99% of tribe members sacrifice themselves in favor of the queen and its few husbands (for details about group selection, see the review of Mitteldorf [28]). It might be suggested that the mole rat is an exception, so to say, some rare mostrosity. However, this is more likely a bright example of the manifestation of a certain general biological law which was noticed by Schopenhauer as far back as two centuries ago [29]: "An individual dies not only of

quite accidental reasons acting in thousands various ways, but it is also doomed to death if this death serves for the preservation of the species."

The modern world of living beings amazes by the diversity and sophistication of extant forms of life. It is quite obvious that this achievement could occur under two conditions: The system is open for further innovations and conservative with respect to previous useful acqirements. The evoluability mentioned by Goldsmith in his review [16] is a high art devised by the biological evolution for its own acceleration. We are presently at the very start of the way to understanding the principles and mechanisms of this extremely complex phenomenon. However, it is already seems quite possible that its result is the supremacy of the interests of the genome over the interests of an individual. This is the only reasonable explanation for the existence of programs written in the genome and useful for this genome but counterproductive for an individual. We suggest that the body's aging program is among them [21].

Possible Structure of the Aging Program

The polemic paper of Bredesen, one of the few advocates of programmed aging, is titled "The non-existent Aging Program: How Does It Work?" [30].

Clearly, any aging program should be a final part of the ontogenetic program, i.e. of the "big biological clocks" described by Dil'man [31] and later by Comfort [2]. To a great regret, it is not still clear where we have our ontogenetic clocks. According to the simplest hypothesis, they are located in the same site as the other, circadian clocks which control the daily 24-hour body rhythm have already been found [4]. This site in birds is located in the epiphyse and in mammals, in the hypothalamic suprachiasmatic nuclei→epiphyse system [4]. One of the key mediators between the circadian clocks and organs is the epiphyseal hormone melatonin. The same hormone might, in principle, also play the role of a mediator for the aging program. It was shown that (1) melatonin production decreases with age by a factor of 7 and (2) melatonin induces the synthesis of a series of antioxidant enzymes and is in itself a powerful antioxidant capable of preventing (while in high concentrations) the experimental stress in mitochondria, in particuler, the oxidation of cardiolipin with reactive oxygen species (ROS), leading to pore opening in the inner mitochondrial membrane and cytochrome *c* release from mitochondria to the cytosol [32, 33].

The idea on the key role of ROS in aging was first advanced by Denham Harman in 1950s [34]. Since then abundant experimental evidence in favor of this idea has been accumulated. In Russia it was taken up as early as 1960s by N.M. Emanuel' and his disciples whose studied ROS as inducers of destructive chain reactions in live cells [35]. Since the principal oxygen consumers in an organism are mitochondria, an idea was raised that the latter are involved in the generation of just those ROS that are responsible for aging [36]. Evidence for this idea was obtained in numerous research (for reviews see [4, 21]). Probably, the most impressive was the recent research of Lambert et al. [37] on ROS generation by mitochondria. It is known that these organelles generate a primary reactive oxygen species, i.e. superoxide radical anion $O_2^{-\cdot}$, by two mechanisms. One of them is operative at the start of the respiratory chain and the other, in its middle. In the first case, the maximum rate of $O_2^{-\cdot}$ formation is quite high, and, therewith, $O_2^{-\cdot}$ releases into the inside of mitochondria. In the second case, $O_2^{-\cdot}$ forms slower and releases into the cytosol. The optimal conditions for the generation of $O_2^{-\cdot}$ by the first mechanism is the rest state, when the adenylic system is present in the form of ATP, and the breathing substrate is succinate. Mitochondria from 12 quite various mammalian species (from mice to caws and babuins) and birds (from quails to pigeons).

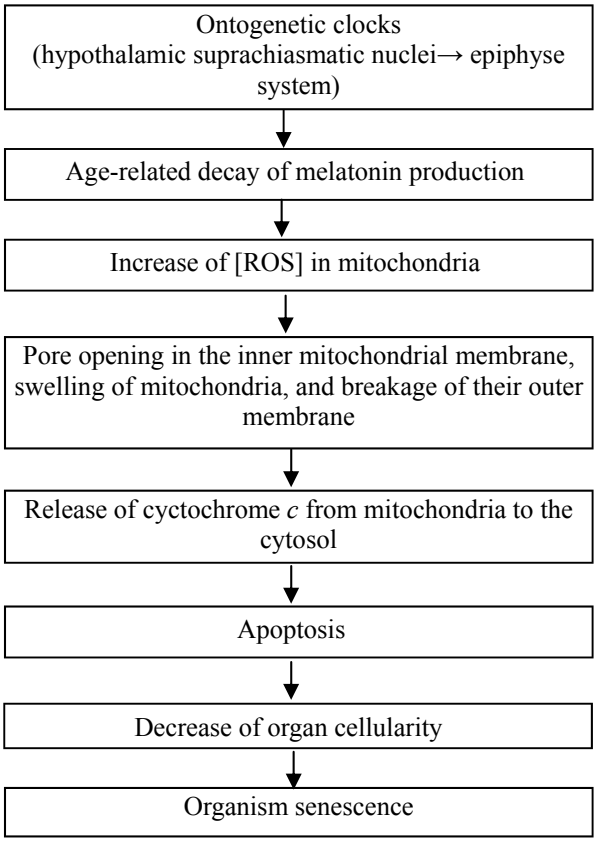
It was found that the maximum lifespan of 11 species of 12 is inversely related to the rate of $O_2^{-\cdot}$ formation by the first mechanism. The only exception was naked mole rats whose mitochondria generate $O_2^{-\cdot}$ even faster than in mice, while the lifespan of the former is longer by an order of magnitude than that of the latter. Damara mole rate which is a close relative of naked mole rat, but did not "guess to establish a monarchy" with all that it implies, fitted well the dependence of lifespan on intramitochondrial ROS production. No correlation was observed between lifespan and the extramitochondrial ROS production by the second mechanism.

In the conclusions to this paper, Lambert et al. [37] "apologize" for mole rats, wondering why it one has fallen out of the above-mentioned correlation. In our opinion, this exception is the strongest evidence in favor of the discovered rule: the more ROS inside mitochondria, the shorter life of aging animals. The case in point that naked mole rat proved to be *the only* (among the species studied by the authors) *representative of unaging beings*. Probably,

intramitochondrial ROS are mediators of the aging program which in the mole rat has mutated somewhere after these ROS.

What is the function of mitochondrial ROS in the 11 species studied by Lambert et al., which does not reveal itself in naked mole rat? As known, mitochondrial ROS act to open large nonspecific pores in the inner mitochondrial membrane, which results in swelling of these organelles, damage of the outer mitochondrial membrane, and release of cytochrome *c* and other proteins causing programmed cell death (*apoptosis*). In its turn, accelerated apoptosis decreases the cellularity of organs, which should result in their functional deterioration, i.e. to senescence. (The idea that aging and organ cellularity are related to each other was first advanced in 1959 by the prominent physicist Leo Scillard [38].) The mechanism of aging outwardly resembles a branch of industry, where the number of workers is all the time decreased but the output plan is left at the same level in the belief that the ingenuity of workers will enhance the labor efficiency.

The above-described hypothetical aging program can be outlined in the scheme [21, 39].



Temporary Dietary Restriction as a Way to Retard the Aging Program

The best evidence in favor of the hypothesis of programmed aging could be provided by a successful attempt of its cancelling. There are some grounds to suggests that such an attempt already gave a positive result as early as 1934, when McCay and co-workers [40–42] managed to extend the lifespan in rats by slightly restricting their diet. This restriction was introduced at early life stages and initially resulted in growth retardation. When the dietary restriction was lifted, the animals rapidly increased in size to reach the norm but lived longer by 70% (males) and 48% (females) than those rats that were fed *ad libitum* all over their life. As to the reason for deaths, a sharp decrease of death rates from pulmonary diseases and tumors was observed. The researchers noted that long-lived animals looked mobile and young irrespective of their age. Their pulse rate was 340 beats per minute, which was about 100 beats below the control. Later the positive effect of a certain dietary restriction on lifespan was demonstrated on a great variety of organisms—from yeast to rhesus monkeys and humans [43, 44]. With the appearance of the Harman's hypothesis on the role of ROS in aging, this effect came to be explained in terms of decreased volume of food oxidized with oxygen and, as a consequence, decreased attendant production of ROS and their caused damage [45]. The inconsistency of this assumption was evident already in early works on dietary restriction, when the same group of researchers found out that the heat production per 1 kg body weight in the experimental rat cohort was higher than in the control [46]. Further research gave direct evidence against the commonly accepted viewpoint.

First, it was found out that it is enough for drosophiles to live starving as long as two days to transform into a "long-liver" to an extent the same as if the fly would be subjected to dietary restriction all over the life [47]. Second, it turned out that not only an excess of food, but also its smell attenuate the geroprotector effect provided by dietary restriction [48]. All these observables are unlikely to be uniquely specific of drosophiles. As far back as 1934, Robertson et al. [49] discovered in their experiments on albino mice that a two-day "fasting" every week is quite sufficient for their lifespan to be extended by 50–60%. Carr et al. [50] observed, again on mice, that the reproductive ability lost by the end of the first day in the case of unrestricted feeding was preserved until at

least the 21st month of life, when mice were limited in feed over the first 11–15 months and then got it *ad libitum*. According to the data of Stuchlikova et al. [51], rats, mice, and golden humsters restricted in feed by 50% over two years, lived longer by 20% than the control. Dietary restriction over one, the first year of life, prolonged the lifespan by 40–60% and over the second year only, by 30–40%.

Further research showed that the effect of dietary restriction is contributed by both the carbohydrate and protein components of food. Therewith, the effect of proteins is associated with one, and only one, amino acid, viz. methionine [52, 53]. Methionine is classed with essential amino acids which are not synthesized in a mammal's body, and their body content depends exclusively on their intake with food. It was found that a diet in which proteins were replaced by a mixture of amino acids containing no methionine not only favors a longer life, but also decreases mitochondrial ROS generation and oxidative damage of mitochondrial DNA [54, 55]. Interestingly, dietary restriction has no effect on the oxidation of a *nuclear* DNA [56], which might be expected by the accepted view of aging as a nonspecific age-related ROS damage of cellular biopolymers and its prevention by a restriction of calorie intake with food.

In our opinion, calorie restriction is perceived by an organism as quite a quite a worrying signal about food shortage. As known, even partial starvation entails decreased fecundity [2]. And this, in its turn, jeopardizes the very existence of the population. To prevent, at least in part, such turn of events, it is sufficient to cancel the aging program, thereby prolonging the reproductive period of the indiviual, i.e. increasing the total number of his descendants. In other words, the impact of dietary restriction on lifespan is only indirectly related to ROS and is a regulatory effect. This is first of all biology, rather than chemistry. That is why such evidently signaling effects as a short-term fasting (or, vice versa, smell of food), and not food shortage (or excess) over the whole life exert a powerful effect on life cycle parameters. It is not accidentally that a temporary dietary restriction (fasting) is better than the constant. The signal can be given for a fairly short time, whereas, as a matter of fact, to live starving for a long time is not quite useful for an organism.[1] Gluttony is characteristic of certain people rather than of animals which do not tend to eat in advance if always have enough food.

The signaling nature of the effect of dietary restriction provides a good rationale for the experiments with methionine. Apparently, the organism determines by itself the amount of available food and, first of all, essential amino acids required for protein synthesis by tracing the level of only one of them, speifically methionine.

Essentially, dietary restriction not only extends lifespan, but also prolongs youth, as already mentioned by the discoverer of this phenomenon McCay. Age-related diseases step back and old animals become indistinguishable from young ones in behavior and even exterior. Quite illustrative in this respect is the research of Weindruch and his group on primates, just reported in [43]. Twenty-year experiments on 76 macaques (adult animals from 7 to 14 years old) showed that a long-term 30% dietary restriction gives the following effects: (1) a sharp decrease of age-related death rate (over 30 years 20% against 50% in the control group fed *at libitum*), (2) exclusion of diabetes from the list of death reasons, (3) halving the death rate from cancer (in macaques this is primarily intestine adenocarcinoma), (4) decrease of the death rate of cardiovascular diseases, (5) decrease of osteoporosis, (6) arrest of the development of such age-related traits as sarcopenia, decline in brain grey matter, alopecia, canities, etc. By the age of 30 years, 80% of the survived control macaques showed any traits of aging , whereas in the experimental group such traits were observed in about as little as 20% of animals.

The experiment on monkeys is still far from completion, and. therefore, we can say nothing about the effect of dietary restriction on the maximum lifespan of primates. At the same time, some evidence on this subject is available for rodents [42, 51]. This evidence shows that the median lifespan (50% death rate) in mice and humsters increases much stronger than the maximum lifespan. The simplest mechanistic explanation of this phenomenon is an arrest (or a strong retardation) of the aging program. Therewith, other ontogenetic programs, first of all body growth, can also be retarded. These phenomena are observed at a sufficiently serious and long-term fasting [40]. However, a more moderate dietary restriction can prolong life not causing growth inhibition [57].

[1] Probably, religious fastings represent a way to prolong life by short-term dietary restrictions. Not excluded that this is an explanation for the known fact that believers live longer than ateists.

Addressed Targeted Delivery of Antioxidants to Mitochondria as a Way to Cancel the Aging Program

What is the level of the cascade of events involved in the aging program (see scheme), where the program is retarded in response to the signal about the threat of starvation? According to Caro et al. [55], we deal here with a decay of intramitochondrial ROS formation and its attendant damage. But if this indeed the case, we can reach the same target by introducing a powerful antioxidant into mitochondria. Our group tried this approach.

The research was based on the two observations we made together with E.A. Liberman and his co-workers in the late 1960s. The first of them is the discovery of an electrical potential difference in the inner mitochondrial membrane (the minus sign is inside mitochondria) [58, 59]. The other observation resulted in the disovery of a new class of ionic species, viz. *penetrating ions*—hydrophobic substances that easily cross biological membranes, regardless of the fact that they have a charged group [59–61]. A typical representative of such ions is methyltriphenylphosphonium. This cation has the phosphorus positive charge strongly delocalized over the three phenyl groups. Therefore, water dipoles cannot sit on the cation and form a shell which could prevent the cation from penetration to hydrophobic parts of the membrane. Since the interior of mitochondria is the only cell compartment negatively charged with repect to its environment (i.e. the cytosole), the penetrating cations, having reached the cell, will selectively accumulate in mitochondria. Such accumulation should be described by the Nernst equation (a 10-fold concentration gradient at the membrane potential $\Delta\psi$ = 60 mV). The $\Delta\psi$ in energized mitochondria is about 180 mV [61], and, therefore, the concentration of the penetrating ion in the mitochondrial matrix should be 1000 times higher than in the cytosole.

These data allowed us to suggest that penetrating ions can be used as electrocarriers for accumulating in mitochondria uncharged substances linked to these cations [62]. This is just our explanation for the role of the cationic group of carnitine in the transport of fatty acid residue inside mitochondria [61, 62]. By electrophoretically concentrating in the inner leaf of the phospholipid bilayer of the inner mitochondrial mambrane, fatty acid acyls and carnitine would be able to act as antioxidants interrupting chain peroxidation of phospholipids and proteins which constitute this leaf. Evidence for this hypothesis is provided by data obtained in our group by Yu.N. Antonenko and A.A. Pashkovskaya. It was found that palmitoyl carnitine prevented from photooxidation of gramicidin inorporated into the planar bilayer phospholipid membrane.

On the border of two centuries the "electrolocomotive molecule" principle was used by M.P. Murphy for the adressed delivery into mitochondria of antioxidants, specifically vitamin E [63] and ubiquinone [64]. The greatest promise was shown by the so-called MitoQ,[2] a substance composed of ubiquinone and decyltriphenylphosphonium cation. The obvious advantage of MitoQ over palmitoyl carnitine or the cationic derivative of vitamin E consists in that its reduced form oxidized during its functioning as an antioxidant can be regenerated by accepting electrons from the respiratory chain. In other words, MitoQ is a recoverable antioxidant. As showed Murphy and coworkers, MitoQ actually accumulates and reduces in mitochondria, protecting them, as well as cell cultures from oxidative stress (for review, see [65]).

We have confirmed the data of Murphy and coworkers on the antioxidant activity of MitoQ. However, it was found that MitoQ rapidly converts into a prooxidant, as its concentration increases. The experiments on rat heart mitochondria (performed in our group under the supervision of M. Yu. Vysokhikh) showed that the $C_{1/2}$ for the antioxidant and prooxidant effects of are 0.3 and 0.5 μM, respectively. Addition of 1.5–2.5 μM MitoQ induces such a vigorous generation of hydrogen peroxide by mitochondria oxidizing NAD-dependent substrates that its rate is the highest on record and close to the respiration rate in the resting state [66, 67] (for the prooxidant activity of MitoQ see also [68–70]). By this reason, MitoQ seems to be an unlikely candidate for antioxidant applications. Therefore, we decided to undertake a search for antioxidants stronger than MitoQ and having a wider gap between the anti- and prooxidant effects.

In 2003 we initiated a project aimed at introducing penetrating ions in practice. This initiative was supperted by a grant of the "Paritet" Charitable Foundation established by O.V. Deripaska. First of all we orderded the synthesis of demethoxyMitoQ. This choice was motivated by the fact that the nematode *Caenorhabditis elegans* lives seven times longer if it has a delection in the gene econding an enzyme

[2] 10-(6′-Plastoquinonyl)decyltriphenylphosphonium.

converting demethoxyCoQ into ubiquinone, as well as in the insuline receptor gene [26]. Experiments with the synthesized substance showed that the gap between its anti- and prooxidant concentrations is, indeed, larger than with MitoQ, but, regretfully, not so much as expected.

Then we turned to plastoquinone, an electron carrier which functions instead of ubiquinone in the photosynthetic electron-transport chain of plants and cyanobacteriae. The reason why in the course of evolution ubiquinone functioning in the mitochondrial respiratory chain was replaced by plastoquinone in the chloroplast photosynthetic chain of the same plant cell may lie just in that ubiquinone ranks below plastoquinone in the antioxidant properties (they were studied in chemical experiments on model systems). Chloplast which in fact forms oxygen always experiences a much stronger oxidative stress than mitochondria which absorb this oxygen. Unlike ubiquinone, plastoquinone has methyl groups instead of methoxyls, and the ubiquinone methyl group is replaced by hyrogen. Such replacements proved to sharply enhance the antioxidant activity. If the anti- and prooxidant concentrations of MitoQ differ less than two times (300 and 500 nM), for the plastoquinone derivative of decyltriphenylphosphonium called SkQ1[3] the respective difference has increased to 32 times (25 and 800 nM) [66, 67].

This result made it clear that we have in our hands a uniquely powerful mitochondria-targeted antioxidant whose action is not complicated by the side prooxidant effect. In this connection I suggested to Deripaska to transform our grant into an investment project aimed at developing a new type of medicines and biotechnological preparations in the basis of SkQ[4] antioxidants. The suggestion was accepted, and the investment project started in the spring of 2005. The project has very soon outgrown the borders of the Belozerskii Research Institute of Physicochemical Biology, Moscow State University, where we performed research in the framework of the initial grant. Now the project team includes research groups from the Biological, Chemical, and Mathematical Mechanical Depatments, the Departments of Basic Medicine, Bioengineering and Bioinformatics, Moscow State University, the Institutes of Bioorganic Chemistry, Molecular Genetics, Developmental Biology, and Biochemistry, Russian Academy of Sciences, Moscow, as well as Moscow Oncology and Cardiology Research Centers, Helmholtz Institute of Eye Diseases, Institute of Polyomyelitis, and Veterinary Academy, St. Peterburg Institutes of Oncology and Cytology, Novosibirsk Institute of Cytology and Genetics, R.W. Johnson Medical School (USA), and Wenner-Gren Institute (Stockholm).

In the search for the best antioxidant, a series of cationic quinone derivatives was synthesized (mostly due to the effort of our chemists G.A. Korshunova, N.V. Sumbatyan, and L.S. Yagizhinskii). In this work we took into account the fact that plastoquinone and the chloroplast electron carrier vitamin K_1, like the "professional" antioxidant vitamin E and unlike ubiquinone (CoQ), contain no methoxy groups. At the same time, vitamins K_1 and E, like CoQ, have in the fifth position of the quinone ring the methyl group which are lacking in plastoquinone. Therefore, we focused on the synthesis of quinone derivatives containing either plastoquinone (SkQ1, SkQ2M, SkQ4, SkQ5, SkQR1[5]) or 5-methylplastoquinone (SkQ3). As the cation we primarily used alkyltriphenylphosphonium with a decyl (SkQ1, SkQ3, SkQ4, DMQ и MitoQ) or an amyl (SkQ5) linker between the cation and quinone. In certain cases, we replaced phosphonium by ammonium cations: methylcarnitine (SkQ2M), tributylammonium (SkQ4), or rhodamine 19.

The synthesized compounds all were tested for their ability to penetrate model membranes. As applied to the bilayer phospholipid membrane, the best penetrateing ability was shown by SkQR1, SkQ1, and SkQ3 (I.I. Severin). Their concentration gradient generates a Nernst diffusion potential with the plus sign in the compartment with a lower cation concentration. The mentioned compounds were then tested (M. Yu. Vysokikh and co-workers) for anti- and prooxidant activity. To measure the antioxidant activity of these compounds, rat heart mitochondria were energized by succinate oxidation and incubated under Fenton reaction conditions (formation of $OH^{\cdot}$ radicals from hydrogen peroxide generated by mitochondria under the action of Fe^{2+}; the reducing agent was ascorbate). The peroxidation of mitochondrial phospholipids, primarily cardiolipine, which occurred under these conditions, was followed by the accumulation of

[3] 10-(6'-Plastoquinonyl)decyltriphenylphosphonium.

[4] Plastiquinone derivatives with a decyl or an amyl linker with the delocalized cation.

[5] 10-(6'-Platoquinonyl)decylrhodamine 19.

malonic aldehyde. The highest antioxidant activity was exhibited by SkQ1 and SkQR1 [66, 67].

These antioxidants were used in experiments on cell cultures and animals. The high fluorescence quantum yield of SkQR1 allowed us to trace its behavior in cells and body. It was shown that in HeLa cells it specifically colors only one organelles, mitochondria, and its highest level on intraperitoneal injection is observed in kidney and liver (data of the groups of B.V. Chernyak and D.B. Zorov) [66, 67, 71].

According to the results of B.V. Chernyak and co-workers, quite low concentrations of SkQ1 and SkQR1 prevent death of human cells in the culture, at low H_2O_2 concentrations. Especially sensitive to these oxidants proved to be the mitochondrial thread–grain transition which occurs in fibroblasts and HeLa cells under the action of H_2O_2 and other apoptogens. The $C_{1/2}$ concentrations which arrest this transition in fibroblasts are 2×10^{-12} M for SkQR1, 1×10^{-10} M for SkQ1, and 2×10^{-8} M for MitoQ, after 2 h preincubation with these compounds. Therewith, MitoQ is unable, even at its optimal concentration, to prevent mitochondrial fragmentation and cell death to the same extent as SkQ derivatives. The desired effect could not be obtained by increasing the level of MitoQ. At concentrations above 1×10^{-7} M, this compound, as well as SkQ derivatives, rather than attenuating the apoptogenic effect of hydrogen peroxide, enhances it.

The efficiency of low concentrations of SkQ1 and SkQR1, revealed in cell experiments, is explained by several factors. First, they have a very high partition coefficient between the hydrophobic and aqueous phases. The octanol–water partition coefficient for SkQ1 is 13000. Further on, the concentration gradient increases another three orders of magnitude due to the $\Delta\psi$ (~180 mV) across the inner mitochondrial membrane and a further order of magnitude due to the $\Delta\psi$ across the outer cellular membrane (~60 mV). Finally, the SkQ1 concentration gradient between the extracellular medium and inner semimembrane layer of the inner mitochondrial membrane can reach really huge values: $13000\times1000\times10 = 1.3\times10^8$. This finding means that if the SkQR1 concentration in the extracellular medium is 2×10^{-12} M, its concentration in the inner semimembrane layes will be 2×10^{-12}–1.3×10^8 = 2.6×10^{-4} M.

Recoverability is one more circumstance explaining the very high efficiency of SkQ antioxidants. Thus, according to our data [66, 67], SkQ1 is readily reduced in the *i* center of complex III of the respiratory chain.

At the next stage of the research we studied the antioxidant activity of SkQ. The experiments were performed on an isolated organ, specifically rat heart. By introducing H_2O_2 into the perfusate we induced cardiac arhythmia. The effect of H_2O_2 was sharply attenuated, if the hearts were taken from rats pretreated daily for two weeks with SkQ1 at a concentration 2×10^{-10} mole per kg body weight (V.I. Kapel'ko and V.L. Lakomkina, Cardiology Research Center) [67, 71]. An attempt was also undertaken to study the effect on SkQ1 on the development of a cardiac pathology in vivo. To this end, artificial myocardial infaction was induced in rats subjected to a similar SkQ1 pretreatment. It was found that SkQ1 at a daily dose of 2.5×10^{-7} mol/kg decreases by 40% the damaged zone of the heart muscle (O.I. Pisarenko and co-workers, Cardiology Research Center) [67, 71].

At our institute, we performed experiments on rats whose one kidney was removed and the blood flow through the other kidney was interrupted for 90 min to induce infaction of this kidney. As a result, 80% of such rats died 5-6 days after the ischemization. The mortality was decreased to 0–20% by a single intraperitoneal injection of 0.5–1 μmol SkQ1 or SkQR1 per kg body weight (D.B. Zorov and E.Yu. Plotnikov) [67, 71]. The same SkQR1 pretreatment radically (threefold) decreased the necrotized zone in rat brain after artificial stroke (D.B. Zorov and N.K. Isaev).

The main goal of most in vivo experiments in the framework of the project was to find out whether SkQ servs as geroprotector. Effects of SkQ1 was studied in three models of accelerated aging (progeria) and normal aging in rodents, fish, drosophiles, and cerio-daphnids. Positive effects of SkQ were observed in mice with X ray-induced progeria (A.G. Ryazanov and co-workers, R.W. Johnson Medical School, USA) and with progeria induced by a point mutation in the gene of mitochondrial DNA polymerase (B. Cannon, J. Niedergard, Venner-Gren Institute, Sweden). In the second case, mice with the mitochondrial DNA polymerase denuded of the ability to correct its own mistakes were used (the aspartate in the exonuclease domain of the enzyme [72]). All mutant mice not treated with SkQ1 died by the 290th day of life with signs of premature aging (gibbosity, body weight loss, decrease in body temperature and loss of ability to maintain it the cold, alopecia, torpor, etc.). Among

mutant mice obtained SkQ1 with drinking water, only one animal died over the same period. Most signs of aging in these mice were either poorly expressed or absent at all [73].

A series of experiments was performed on rats of the OXYS strain suffering progeria caused by a constant oxidative stress. This strain is characteristic in that such age-related diseases as cataract, retinopathy, osteroporosis, thymic involution, decreased sex motivation in males, and memory decay develop already a few months after birth. It was found (N.G. Kolosova and co-workers) that the development of such signs of aging is sharply retarded, if animals were given food containing 50–250 nmol/kg per day. Eye drops containing SkQ1 (2.5×10^{-7} M) cured already developed cataract and retinopathy within 1.5 months. Evidence for the efficiency of such therapy of cataract was obtained in experiments on the usual Wistar rats. As to retinopathy, Wistar rats seldom live long enough for this desease to develop, and, therefore, we had to change the object. Collaborative research of our institute (I.I. Senin and co-workers) and the Skryabin Veterinary Academy (E.P. Kopenkin and L.F. Sotnikova) showed that SkQ1 drops are effective against retinopathy in dogs, cats, and horses (a total of 271 animals who failed to react to routine treatments were treated with SkQ1). The most evident positive effect was observed in treatment of inherited retinal dysplasia (a radical improvement was observed in 67% of cases) and its secondary degeneration (54%). In the case of progressive retinal degeneration, SkQ1 proved to be less effective (29%). Of 89 completely blind animals, 67 started to see again after SkQ1 treatment for two–three weeks [67, 74].

There positive results are in contrast with the failure of researchers of Murphy's group, who attempted to treat inherited retinopathies in mice by means of MitoQ [75]. Apparently, this result is explained by the fact that the effective concentrations of MitoQ, causing the antioxidant effect, are too close to its prooxidant concentrations. The authors of [75] also suggested that the studied retinopathies are at all insensitive to antioxidants.

To complete this series of research, we turned to aftificially induced eye diseases in animals, namely experimental uveitis (I.I. Senin's group) and glaucoma (V.P. Erichev and co-workers, Helmholtz Institute). Both these diseases are known to be associated with a heavy oxidative stress [76, 77], and they both proved to be treatable with antioxidant SkQ1 drops [67, 74]. Experiments on the therapy of uveitis were especially spectacular. This autoimmune disease was induced by immunization of rabbits with a photoreceptor cell protein arrestin. One eye of the blind animals was instilled with SkQ1 (four times a day). In a few days, the treated eye recovered, whereas the untreated eye not. The same procedure prevented the development of uveitis, if the drops were applied over the immunization period. Therewith, the success was reached in 100% of cases both in preventing uveitis and in treating the already developed disease. P.P. Filippov (Belozerskii Institute of Physicochemical Biology) and E.N. Grigoryan (Kol'tsov Institute of Developmental Biology) managed to simulate the effect of SkQ1 in the treatment of retinopathies. In roller cultures of eye fundus, SkQ1 decreased eightfold the transformation of the pigmented epithelium cells of the retina into phagocytes which destroy this tissue [74].

Extensive research on the effect of the SkQ1 antioxidant on the aging process was performed at the Petrov Institute of Oncology (St, Petersburg) by the group headed by the President of the Russian Gerontological Society V.N. Anisimov. It was shown in mice experiments that SkQ1 (0.5, 5, or 50 nmol/kg per day) appreciably decreases the age-related mortality. This effect is especially impressive on the first 20% of the died animals: Their lifespan was increased 2.5-fold. With 50% of the died animals, this effect was 100%, and with long-lived mice (the last 20% of the studied cohort), only 5–15%. Thus, the so-called rectangulation of the mortality curve is observed. The same regularity was revealed with invertebrates, specifically drosophile (E.G. Pasyukova and co-workers, Institute of Molecular Genetics), ceriodaphnia crystacean (O.F. Filonenko, Biological Department, Moscow State University), *Nothobranchius furzeri* fish (K.M. Shidlovskii, Belozerskii Institute of Physicochemical Biology, Moscow State University), and mycelium of *Podospora anserina* fungus (M. Yu. Vasokikh, the same institute), whose median lifespan increased considerably under the action of SkQ [78].

It was shown that SkQ1 not only affects the lifespan; in doing so, it also improves the quality of life. Thus, for example, the regular estrous cycles ceased in 70% of control mice by the 22nd month of life, continued at the same level that in young aminals, if they obtained SkQ1 with drinking water. The

antioxidant changed sharply the pattern of mortality reasons. It was found that SkQ1 much decreases the mortality of infections, which increases in its absence with age-related decay of immunity (this effect can be compared with the SkQ1-induced inhibition of the involution of the thymus and follicular spleen compartments in OXYS rats, since these organs generate the key components of the immune system: T and B lymphocytes [79]). Decreased mortality rate of certain forms of cancer is also observed. At the same time, SkQ1 failed against breast cancer which proved to be the main reason of mortality of animals treated with SkQ1 (all experiments were performed on females). Special experiments on HER-2 mice , susceptible to breast cancer showed that SkQ1 scarcely affects the mortality of these animals which died before one year of age.

It was shown (B. P. Kopnin and co-workers, Oncology Center, Moscow) that very small amounts of SkQ1 (5 nmol/kg per day) essentially increase the lifespan of mice deficient of the p53 protein and, as a result, suffering the oxidative stress. Such mice die largely of lymphomas by the 250th day of life. The survivorship curve for animals obtained SkQ1 with drinking water shifts markedly to the right. Therewith, a sharp decrease in the ROS level in spleen cells was observed [80]. The same effect was revealed, when mice were treated, instead of SkQ1, with the traditional (not mitochondria-targeted and nonrecoverable) antioxidant *N*-acetylcysteine. However, the dose of the latter was 6 mmol/kg per day, i.e. 1.2 mln times higher than that of SkQ1 (*N*-acetylcysteine in these quantities is toxic on a long-term basis). It was also found out that p53-deficient human colon carinoma cells are much more tumorogenic, when implanted in thymus-free mice. Such an enhanced aggressiveness of tumor cells was completely blocked by SkQ1 [80].

The table summarizes the results of our in vivo animal research on SkQ1 and SkQR1. As seen, 22 various signs of aging step back under the action of SkQ, which allows these substances to be classed with powerful geroprotectors. The feature of the geroprotector action of SkQ1 is that it not only and not so much extends the maximum lifespan, but improves its quality at the second half of the life cycle. In other words, it prolongs youth.

Comparison of the Effects of Dietary Restriction and SkQ

The effects of dietary restriction and SkQ antioxidants have much in common. In both cases, rectangularization of survivorship curves and a sharp decrease of early mortality rates are observed, and the medium lifespan increases much more than maximum. These factors work not not only, and not so much, as to prolong life as such, as to prolong a healthy young life. There factors both are very effective in living beings quite different in their systematic position (dietary restriction works well in yeast, insects, and mammals, and SkQ, in mycelium fungi, crustaceans, insects, fish, and mammals). The effect of both factors is clearly pleiotropic in nature, i.e. they cause response

Effects of SkQ antioxidants

Deacreases age-related mortality	Inhibits loss of sex motivation in males
Inhibits development of retinopathies and cures already developed retinopathies	Prevents cessation of estrous cycles in females
Inhibits development of cataract and cures already developed cataract	Stimulates wound healing
Inhibits development of uveitis and cures already developed uveitis	Inhibits osteoporosis
Inhibits development of glaucoma	Inhibits mouse alopecia
Decreases zone of myocardial infarction	Inhibits loss of whiskers in mice
Lifts *cardiac arrythmia*	Inhibits graying at X-ray-induced progeria
Decreases stroke zone	Inhibits development of hypertension
Prevents death of renal infarction	Inhibits development of progeria induced by mutation in the mitochondrial DNA polymerase
Inhibits age-related involution of thymus and follicular spleen compartments	Prevents age-related anemia
Inhibits development of lymphomas and certain other cancers in p53-deficient animals	Prevents age-related decrease of blood lymphocyte level

from a variety of physiological body systems, and, moreover, these are generally one and the same systems. Cardiovascular diseases, osteoporosis, vision disorders, and certain types of cancer step back, graying and hair fall, as weel as age-related depression do not occur.

Contradictory data concerning effects of dietary restriction on sarcopenia and immune responses were obtained. Some authors state that such exposure adversely affects both the muscle system and immunity[6] (see the reviews [82, 85]). On the other hand, Weindruch and co-workers [43] reported the lack of sarcopenia in monkeys and McCay and co-workers [40], resistance to pulmonary diseases in rats subjected to dietary restriction (cf. sharp decrease of age-dependent inhibion of the involution of the thymus and follicular spleen compartments in rats exposed to SkQ [67, 79]). At the same time, the effects of dietary restriction and SkQ on wound healing are opposite to each other: staring life inhibitedinhibited [82], while the antioxidant SkQ1, by contrast, stimulated [21] this process. Dietary restriction decreases body temperature and inhibits growth, which was not observed with SkQ. These data rule out such a trivial interpretation of our data as the assumption that SkQ1 decreases animal food intake, say, by decreasing appetite. Direct measurements on mice treated with SkQ1 revealed no decrease of food intake.

The fact that dietary restriction adversely affects some vitally important parameters is not surprising. As mentioned above, animals do not tend to overeat even if they are not restricted in food. Usually they eat as much as needed for their organism. Therefore, long-term dietary restriction entails any disorders in vital functions. It is also clear that such disorders are the more probable the longer is starvation. We already mentioned that long-term and continuous dietary restriction is not at all necessary for the geroprotector effect. This explains the controversy in data on the effect of dietary restriction on lifespan and organism state. In cases when dietary restriction was not too strong and not too long, positive effects were observed, whereas when gerontologists overdid the restriction, unfavorable side effects took place. Thus, it is commonly accepted that long-term dietary restriction decrease the frequency of estrous cycles (sometimes until they cease completely) [82], but as fas back as 1949 Carr and co-workers showed that temporary dietary restriction with its subsequent cancellation, vice versa, prologs estrous cycles and favors their preservation until an extreme old age [50]. It will be remembered that the same effect was observe with SkQ [78]. In principle, there is no need to starve all over the life, if starvation prolongs life by switching off the aging program (vide supra). However, there is a probablity that too weak or delayed dietary restriction will only partially retard the program, and the geroprotector effect will turn weak. The use of SkQ1 appears not to pose such a threat, since SkQ1 acts in such low concentrations that no adverse effects have still been observed [21, 67].

One more circumstance to be borne in mind when considering undereating as a geroprotector for humans. Actually, if dietary restriction is a signal to warn about death of starvation, then the organism should respond not only by prolonging life so that to compensate for the decay of birth rates in lean years. Quite possible are other responses, and some of them may prove not as attractive as extension of a healthy life. For example, it was noted that a hungry mouse, once found itself in a squirrel cage, does not want to leave it and makes from 6 to 8 km over night (at normal feeding this distance is always shorter than 1 km) [82]. This effect is by no means explainable by starvation-induced exhaustion and muscle weakness. More likely, we deal here with one more response on the starvation signal: extreme anxiety and attempted scanning as large space as possible in a search for food. Parafrasing the Strugatsky's novel "Monday Begins on Monday," we deal here with a mice "unsatisfied stomachically." Had this effect been characteristic of SkQ, we would have observed enhanced food intake by animals obtained SkQ, which is not the case. An impression arises that the use of SkQ is a "purer" way to switch off the aging program, not overburdened with undesirable side effects.

From *Homo sapiens* to *Homo sapiens discatenatus*

There is at least one application field, where dietary restriction will unlikely to replace SkQ. Our research gives us grounds to suggest that SkQ1 is quite a promising agent for creation of new-generation medicines acting on aging processes as antioxidants specifically targeted at mitochondria. Therewith, SkQ

[6] Fernandes and co-workers [81] noted that dietary restriction, especially in a young age, inhibits interleukine secretion by macrophages as a response to bacterial polysaccharides. In its turn, this adversely affects resistance to sepsis and peritonitis [81, 82]. According to Gardner [83, 84], partial starvation abolishes resistance to influenza virus.

acts not only as a geroprotector, as evidenced by expeiments on young animals with artificially induced diseases (cardiac arrhythmia, cardiac and renal infarctions, stroke, wounds, tumors, etc.). Surely, we can suggest that SkQ simply "cleans the durtiest place in a cell" and thus exerts a certain nonspecific favorable ffect on various aspects of its vital functions. However, such explanation is unlikely to be true. In this case, it remains unclear why during their evolution the plants that synthesize separately plastoquinone and the penetrating cation berberine, have not formed their combination capable of removing ROS from the inferior of plant mitochondria experiencing a higher "oxygen threat" than animal mitochondria, since the cytosol in plant cells are saturated with oxygen formed by chloroplasts.

An alternative possibility consists in that the program triggering aging as a slow phenoptosis is also used in other deadly programs which trigger rapid, or acute, phenoptosis. Here we come to a fundamental question of biology as to how genetic programs couterproductive for an individual can exist at all. Darwin [19] and Wallas and Weismann [12] suggested as early as XIX century that the death of an individual may be altruistic for a family or community. In 1964 Hamilton published a series of two papers, titled "Genetic Evolution of Social Behavior" [86], and in 1976 Dowkins published the book "The Selfish Gene" [5], in which the author developed and popularized the Hamilton's concept, concluding that "the main selection unit is a gene rather than an individual." In essence, the issue here is already not social well-being, but the "dictatorship" of the genome, the only self-reproducing biologic structure whose preservation, development, and expansion has taken priority over the well-being of an individual or a group of individuals. In terms of this concept, organism is nothing but a machine serving for interests of the genome.

Recently we have formulated a principle called the "samurai" law of biology: "It is better to die than to be wrong" or, in more detail: "Complex biological systems (from organelles and и выше) are equipped with programs of self-elimination, which are actuated when the given system appears to be dangerous for any other system occupying a higher position in the biological hierarchy" [4]. Together with the "genome dictatorship" concept, this law implies that any critical state of an organism, when it no longer guarantees safety of its genome and, in the case of convalescence, can generate offspring with a changed genome, should be a signal for the organism's self-elimination, i.e. phenoptosis. According to Monsieur Bahys, one of the heroes of Molière's comedy "Love is the Best Doctor": "It is better to die by all rules than to recover agains the rules."

It seems quite possible that the mechanisms of rapid phenoptosis and slow phenoptosis (aging) are mediated by intramitochondrial ROS at early stages of the process. If this assumption is valid, then the positive effect of SkQ antioxidants not only on aging, but also on a great variety of acute pathologies in both young and oil organisms, can be explained in terms of neutralization of these ROS and switch-off of rapid phenoptosis.

It is not excluded that SkQ can serve as a tool in the "rise of machines," an attempt of *Homo sapiens* to do away with genome tyranny and to cancel those genome-dictated programs which are useful for the genome but unfavorable for an individual. Acute phenoptosis of a human fallen in a critical state and committed a biochemical suicide according to the above-mentioned "Bahys rule" is as a harmful atavism as aging. Probably, there are other analogous genetic programs couterproductive for the organism. Their cancellation would symbolize conversion of a human into *Homo sapiens discatenatus* (from Latin *catena,* which means chains or irons), which would be the highest achivement of the medicine of the XXI century [21].

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