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# HOW AND WHY WE AGE

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**Abstract—**After performing the miracles that take us from conception to birth, and then to sexual maturation and adulthood, natural selection was unable to favor the development of a more elementary mechanism that would simply maintain those earlier miracles forever. The manifestations of this failure are called aging. Because few feral animals age, evolution could not have favored a genetic program for age changes. Natural selection favors animals that are most likely to become reproductively successful by developing better survival strategies and greater reserve capacity in vital systems to better escape predation, disease, accidents, and environmental extremes. Natural selection diminishes after reproductive success because the species will not benefit from members favored for greater longevity. The level of physiological reserve remaining after reproductive maturity determines longevity and evolves incidental to the selection process that acts on earlier developmental events. Physiological reserve does not renew at the same rate that it incurs losses because molecular disorder increases at a rate greater than the capacity for repair. These are age changes, and they increase vulnerability to predation, accidents, or disease. Failure to distinguish aging from disease has not only blurred our efforts to understand the fundamental biology of aging, but it has profound political and economic consequences that compromise the field of biogerontology. Changes attributable to disease, or pathological change, can be distinguished from age changes for at least four important reasons. Unlike any known disease, (1) age changes occur in every human given sufficient time, (2) age changes cross virtually all species barriers, (3) no disease afflicts all members of a species only after the age of reproductive success, and (4) aging occurs in all feral animals subsequently protected by humans, even when that species probably has not experienced aging for thousands or millions of years. The resolution of age-associated diseases will not advance our knowledge of aging, just as the resolution of the diseases of childhood did not advance our knowledge of childhood development. We have failed to convey that greater support must be given to a question that is rarely posed. It is a question that is applicable to all age-associated diseases, and its resolution will also advance our fundamental knowledge of aging: "Why are old cells more vulnerable to pathology and disease than are young cells?" During the first half of this century it was believed that because cultured normal cells were immortal, aging must be caused by extracellular events. Thirty-five years ago we overthrough this dogma when we found that normal cells do have a limited capacity to divide, and that age changes can occur intracellularly. We also observed that only abnormal or cancer cells are immortal. Normal cells are mortal

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because telomeres shorten at each division. Immortal cancer cells express the enzyme telomerase that prevents shortening. Recently, it was discovered that when the catalytic subunit of the telomerase gene is inserted into normal cells they become immortal. 

**Key Words: aging, senescence, evolution, disease, telomere, telomerase, cell culture, cytogerontology**

## INTRODUCTION

I WANT TO THANK Drs. Richard Falvo and Andrjez Bartke and the Program Committee for their kind invitation to address you this evening. The assignment given to me is to present an overview of the field of aging and the directions that it might take in the future. For me to give a balanced overview of all of biogerontology and its future directions in the allotted time would be presumptuous. Consequently, I will restrict my remarks to an attempt to answer these two questions: "What is biological aging?" and "How does it happen?"

Many years ago the most cogent expression of why aging occurs was made in the following statement:

"It seems strange that after performing the miracles that take us from conception to birth, and then to sexual maturation and adulthood, nature was unable to devise what would seem to be, a more elementary mechanism that would simply maintain those earlier miracles forever. Nature has not done this, and the reason for her failure is an enigma."

Like many of you here, I have given this question considerable thought, and as my knowledge of the field has increased, my ideas about the origins of age changes have evolved. What I will present is my current thinking and, I am sure that this, too, will change.

One change in my thinking is that we probably do know more about the causes of age changes than we ever have. The common argument that there are as many theories of aging as there are biogerontologists is nonsense. All theories of aging are derivative of one fundamental idea, and that is that aging is simply an increase in molecular disorder.

Before addressing this idea, I want to make clear what I believe is a crucial distinction that must be made between the concepts of aging and longevity determination. I do not believe that they are the same. And, I believe that the failure to distinguish between the two concepts has led us, and is leading us, into much confusion.

## AGING AND LONGEVITY DETERMINATION

Longevity determination, as distinguished from age changes is, in my view, indirectly determined by the genome. I believe that aging is a stochastic process that occurs after reproductive maturity and results from increasing molecular disorder. I do not believe that age changes are directly programmed by the genome.

My conclusions are based on the following reasoning:

Species survival depends on a sufficient number of members of that species living long enough to reproduce and, if necessary, to raise progeny to independence. The verity of this premise seems obvious, because if animals are unable to reach sexual maturity, they will not reproduce and the species will vanish.

The best way to ensure survival to reproductive success is for natural selection to favor animals that have greater physiological reserve in vital organs and, thus, more capable of surviving predation, disease, accidents, and environmental extremes. Prey survival will occur by

natural selection, because as a predator becomes more skilled in capturing prey, the prey that survive will do so by developing better avoidance techniques. And the reciprocal. In a harsh environment animals whose vital systems have a large overcapacity are better suited to survive. That is, animals that develop greater reserve capacity in their vital systems, a more efficient healing process, faster sensory responses, or greater strength or speed are better able to escape predators, and survive disease, accidents, and harsh environmental conditions. The favored animals will have developed "redundant capacity" or greater "physiological reserve."

Greater, or redundant physiological capacity increases the chances for animals to survive long enough to achieve reproductive success, just as redundant vital systems in complex machines, like space vehicles, better ensures that they will achieve their goals. Once animals achieve reproductive success, the excess physiological capacity, like that engineered into a space vehicle, allows each to continue beyond the vital goal. Further survival of the animal beyond sexual maturation and the space craft beyond its primary mission is determined by the level of excess capacity present at the time each goal was reached (Hayflick, 1996).

Because survival long beyond reproductive success has diminished value for the survival of a species, the forces of natural selection diminish after animals achieve reproductive success. Energy is better spent on guarantying reproductive success than it is for increasing individual longevity. After reproductive success the forces of natural selection do not favor increased longevity. However, after reproductive success an animal has the potential to survive for a period of time determined by the level of excess physiological capacity reached at sexual maturation (Hayflick, 1996). And, that is what I call longevity determination. It is not an absolute number. The level of excess physiological capacity reached at the time of sexual maturation determines the potential for continued longevity. It is conceptually different from aging and is independent of the aging processes.

The molecular order achieved from conception to sexual maturation becomes more disordered over time. Physiological reserve does not renew at the same rate that it incurs losses, thus molecular disorder increases. Disorder increases in spite of the presence of repair processes because repair processes themselves incur molecular disorder and are unable to keep vital processes in perfect repair forever. The increasing molecular disorder increases the vulnerability of the animal or human to predation, accidents, or disease.

Clearly, the developmental events that lead to the survival of animals to reproductive success are determined genetically. Therefore, the survival of animals beyond sexual maturation is determined only indirectly by the genome. The state of survival beyond reproductive success might be regarded as a period of "coasting" or "free wheeling," that is, developmental processes have ended, and the capacity to maintain vital systems declines. The length of this postreproductive period plus the time taken to reach sexual maturation can be viewed as the two components of an animal's longevity (Hayflick, 1996).

The unrepaired molecular disorder that occurs after reproductive success and that fails to maintain the system produces what we recognize as age changes, and increases the probability of dying. Because life occurs in an open system, the molecular disorder that occurs cannot be thought of as a result of the Second Law of Thermodynamics, that is, increasing entropy.

By this reasoning the aging of living things that are incapable of perfect repair is not unlike the aging of everything else in the universe, including the universe itself. Absent perfect mechanisms for repair, molecular disorder increases. The disorder might occur passively by simple decrements in the energy necessary to maintain molecular order or actively through, for example, the action of oxygen free radicals.

It is for these reasons that I do not believe that there exist genes that have evolved to directly

drive age changes. Furthermore, as I will describe subsequently, it is unlikely that feral animals ever live long enough for genes that govern age changes to have evolved.

I do believe that there are genes whose action will increase physiological capacity or reserve and thus indirectly increase the potential for greater longevity.

Nevertheless, there are animals that seem not to age at all or, if they do age, the processes occur at the limits of our ability to detect them. I refer to that class of animals who generally do not reach a fixed size in adulthood. Examples include some tortoises, sport and deep sea fish, and the American lobster. It is highly questionable whether these animals age at all, and the reasons for this remain a challenge to biogerontologists.

## AGING IN FERAL ANIMALS

I would like to address the question of aging as a peculiar human phenomenon. I have pointed out earlier that aging is an artifact of human civilization (Hayflick, 1996).

There is a good argument for the belief that extreme manifestations of aging are unlikely to occur in wild animals because they rarely live long enough to experience them. The same is true for prehistoric humans. Age changes do not cause sudden death after reproductive success because it would be prohibitively costly in energy to evolve a system in an animal that would cause it to die precisely on the day that its progeny become independent.

It is analogous to the manufacture of a cheap watch. The owner of a cheap watch company who guarantees that his watches will function for one year will quickly fail in business if the one-year guarantee cannot be met. To achieve this goal, the manufacturer makes certain that the materials used and the workmanship employed will result in a product that lasts at least one year. But, what the watchmaker and natural selection do not do is to engineer into the system an expensive mechanism that will ensure that the watch fails on the 366th day, or that causes the animal to die immediately after reproductive success. This is not done because the costs of doing either will drive the cheap watchmaker out of business or cause the animal to expend an enormous amount of energy on a process that has no species-survival value.

The class of animals generally referred to as “big bang animals,” represented by the Pacific Salmon and the marsupial male rat, may appear to be an exception to this notion. However, it is more likely that the deaths that occur after reproductive success in these animals is the result of their unique expenditure of enormous amounts of energy that precedes mating. It is questionable whether the biological changes that precede their deaths are age changes.

Redundant physiological capacity is common in human vital organs. However, there is a price to pay for redundant systems. That price is the expenditure of more energy during development to make the redundant systems that are necessary to better guarantee survival to reproductive success.

Some biogerontologists contend that there is a “trade-off” between reproductive effort and lifespan. They maintain that high reproductive capacity correlates with a short lifespan, and low reproductive capacity correlates with a long lifespan.

Animals make a “trade-off” between growing up fast and then producing many offspring—a strategy that better favors the preservation of the organism’s genes—vs. survival of the animal for a longer time, which may or may not benefit reproductive success.

The energy costs of a perfect repair system are so high that it would put its possessor at an evolutionary disadvantage compared with an animal that puts less energy into repair and more into fueling greater fecundity. For example, if an animal has a large mortality rate in the wild, like many species of birds, then it is much better for the birds to invest their energy in growing

up fast and producing many offspring than it would be to invest the energy in strategies that might keep the birds alive for a longer time. In fact, this principle recently has been observed to occur in feral guppies.

A general rule of thumb is that the larger the proportion of resources allocated for reproduction, the lower is the chance that members of that species will live a long time.

A high adult mortality rate favors the evolution of a life style in which there is an early and high reproductive effort. The opposite strategy, in which energy is invested in a later reproductive effort, is one that has been adopted by humans and other animals (like elephants), where resistance to environmental hazards, or better adaptation to the environment, has reduced the likelihood of deaths from accidents, disease, predation, or starvation. Using this strategy, slow growth rate and a slow reproductive rate results in increased Darwinian fitness and, indirectly, in a longer lifespan.

Stated another way, natural selection trades those traits that favor greater longevity for those traits that favor enhanced early fecundity. The force of natural selection then declines with age, an idea first emphasized by the late Sir Peter Medawar.

## AGING IS AN ARTIFACT OF HUMAN CIVILIZATION

Engineers have a term to describe the average period of time that they expect a mechanical device to survive. It is called the "mean time to failure." The mean time to failure of a cheap car might be two years before some major repair might be needed. A more expensive car might have a mean time to failure of five years. "Mean time to failure" refers to a future time when half of a group of identical objects will stop functioning. It is identical to life expectation in humans where the "mean time to failure" of today's babies is about 75 years.

If longevity is determined by our genes, albeit indirectly, and aging is not, then why do we age?

It is difficult to see how evolution could select for a process like aging when few, if any, animals ever lived long enough to participate in the selection process.

Humans are the only species in which a large number of members usually experience aging. Aging in numbers proportional to those seen in humans simply does not occur in feral animals. It occurs only in the animals that humans choose to protect. Furthermore, animals that reach old age are not essential for the survival of any species. Humans for example, had a life expectation at birth of 30 years or less for more than 99.9% of the time that we have inhabited this planet. Prehistoric human remains have never revealed individuals older than about 50 years of age. There appears to be no selective advantage favoring the survival of old animals or old humans.

Finally, members of exotic wild-animal species, who for millions of years have not experienced aging, reveal age changes when protected by humans who keep them as pets or house them in zoos. It would be difficult to explain how evolution could have selected for a process like aging that could be made to appear in all members of a species after its expression was suppressed for millions of years.

Indeed, biological aging may have arisen as a phenomenon coincident with the appearance of the human species. The only animals that experience significant age changes are humans or the animals that we choose to protect. Because humans, unlike feral animals, have learned how to escape the causes of death long after reproductive success, we have revealed a process that, teleologically, was never intended for us to experience. One might conclude, therefore, that aging is an artifact of civilization.

It is for this reason that the late George Sacher proposed that we have been asking the wrong

question. Instead of asking "Why do we age?" the right question should be "Why do we live as long as we do?" By asking that question we might reorder our thinking and design experiments in a way in which more fundamental information will be obtained. I think this is a useful approach, and I hope that more biogerontologists will come to realize the subtle but important reason for asking this better question.

## DISEASE AND AGING

I want now to turn to another important conceptual distinction. That is the distinction that I think must be made between age changes and disease. The two are not the same. And failure to recognize this has not only blurred our understanding of aging, but it has had profound political and economic consequences that, in my view, have negatively impacted the field of biogerontology.

My concerns are based on what I believe to be the mistaken assumption that an understanding, or even the resolution, of age-associated diseases will advance our knowledge of the fundamental aging processes. This dubious assumption is a result of the failure of many biogerontologists, and the general public, to distinguish disease or pathology from aging. The distinction between disease and the processes of aging is central to an understanding of why I believe that the resolution of, for example, Alzheimer's Disease, will tell us little, if anything, about the fundamental biology of age changes. In fact, the resolution of the leading causes of death in old age—cardiovascular disease, stroke, and cancer—also will not advance our knowledge of the aging processes.

Put another way, the resolution of age-associated diseases will advance our knowledge of the aging process to the same extent that the resolution of pediatric-associated diseases, such as poliomyelitis, advanced our knowledge of childhood development. That is, with the resolution of poliomyelitis, no advancement in our understanding of human development occurred at all. To understand why, I must tell you why I believe that aging, like childhood development, is not a disease.

Changes attributable to disease can be distinguished from age changes for at least four important reasons. First, unlike any known disease, age changes occur in every human given sufficient time. Second, unlike any disease, age changes cross virtually all species barriers. Third, unlike any disease, age changes occur in all members of a species only after the age of reproductive success. Fourth, unlike any disease, aging occurs in all animals removed from the wild and protected by humans even when the species has not experienced aging for thousands or even millions of years.

How many conferences or publications on the biology of childhood development are dominated by discussions of childhood diseases in the belief that their study, or even their resolution, will provide greater insight into how a child becomes an adult. If we want to know how and why an adult ages why is it necessary to study their diseases? The answer often given is that the study of disease or pathology will provide us with useful information about the biology of aging in nondiseased normal cells. This is a valid point, but it is also a two-way street. No one would know what pathology is without knowing what is normal. And the present situation in biogerontology, which emphasizes disease and not age changes in the mind of the public, is really the root of the problem. It is virtually impossible to raise funds for research on aging, because in the mind of the public no one suffers or dies from aging. We suffer and die from the diseases that occur during the aging process. Yet, in respect to aging, it is the usual losses in physiological capacity that increase our vulnerability to disease and ultimately to death.

A distinction must be made between age changes and the pathology of disease, because our failure to do so has resulted in the present circumstances where the funds available for research in biogerontology heavily favor research on age-associated diseases with comparatively trivial support for fundamental research in biogerontology. Perhaps the best example of this, of several that could be given, is the fact that more than 50% of the present budget of the National Institute on Aging in the United States is spent on Alzheimer's Disease studies. I think that the present disproportionate amount of funds committed to studies on the fundamental processes of aging is unacceptable. At least, in the case of Alzheimer's Disease, its resolution will not only have a trivial impact on human life expectation, but it will not advance our knowledge of the fundamental biology of aging. What we have failed to convey is that greater support must be given to a question that is rarely posed. It is a question that is applicable to all pathologies and diseases of the elderly. And, it is a question whose resolution will also advance our fundamental knowledge of aging. It is this: "How do old cells differ from young cells and why are they more vulnerable to pathology than are young cells?"

We frequently talk about aging as "normal aging," but to do so implies that there is a condition of "abnormal" aging. This, of course, is absurd. There are many age changes that do not compromise health or increase the likelihood of death. For example, no one has ever died of wrinkled skin, grey hair, or menopause.

To carry this argument to its logical conclusion, I would be willing to defend the position that no one over some arbitrary old age say, 80, has or will die from what was written on his or her death certificate. I would defend the belief that all persons over age 80 die from the usual increase in molecular disorder or age changes that they have lived long enough to incur. Those changes simply have increased their vulnerability to whatever was written on their death certificates. This would even apply to most accidental causes of death where diminished eyesight, hearing, or increased reaction time was the direct cause of the accident.

One must ask what would we die from if all causes of death currently written on death certificates would be resolved. After all, that is the goal of all biomedical research. Put more starkly, our goal should be to drive the NIH, every biomedical research facility, hospital, and medical center out of business and to make our own jobs superfluous. I cannot imagine anyone disagreeing with that goal, although it is rarely discussed.

I am old enough to remember when many people died from natural causes. Few, if any people die from that cause today. One of the greatest triumphs over a leading cause of death in the 20th century has become so marginalized that, as we approach the end of this millennium, no one has asked the obvious question: Who cured natural causes, and how was it done?

Despite the magnitude of this achievement, I can find nowhere in the scientific literature a description of how "natural causes" were resolved. The extraordinary modesty of the discoverers of the cure, if not the etiology, of "natural causes," is awesome. The mystery deepens when one considers that this monumental achievement occurred without grant support.

The search for the etiology and resolution of deaths attributable to natural causes is not a silly exercise because that is precisely one of the most important questions that can be asked in biogerontology. It is important because if all, or most, causes of death now written on the death certificates of the elderly are resolved, we will be faced with the reality of how death occurs in the absence of disease. The resolution of deaths attributable to the current causes appearing on the death certificates of the elderly will not result in immortality. It will result in the revelation of the true underlying causes of all such deaths. That is, the inexorable loss of physiological capacity in vital organs, which is the hallmark of aging. We will then have to invent a new vocabulary to describe these new causes of death.

The term “natural causes” represents that category of deaths that are attributable to the absence of disease and the presence of overwhelming age changes recognizable as loss in physiological capacity.

I would suggest that the resolution of “natural causes” as a leading cause of death occurred because physicians have felt that to write “natural causes” on a death certificate, even when the cause of death is truly unknown, is an admission of ignorance and, hence, undesirable in an era of presumed scientific enlightenment. Thus, in the United States, cardiac arrest, stroke, pulmonary infarct, cancer, or some other guessed-at cause is thought to be more professionally acceptable than to admit that the cause of death is natural or unknown. And, the real cause of death is almost always unknown for very old people. The impact that this nonmedically based, sociologically determined phenomenon has had on the statistics of true causes of death in the United States in the past 50 years can only be speculated upon. How much have we been misled to believe that some disease causes of death have increased merely because those causes have come to replace what was formerly called “natural causes?”

The resolution of all causes of death currently written on the death certificates of those over age 65 will only result in an increase in life expectation at birth of about 15 years. What needs to be better understood is that an increase in our knowledge of how age changes occur does not put a 15-year limit on what is possible.

## CYTOGERONTOLOGY

In this, the final part of my presentation, I want to discuss some of the recent advances in cytogerontology or cell aging.

I refer to those advances made in the last few years that have provided new insights at the molecular level into why normal cells have a limited capacity to replicate and what that might be telling us about aging and longevity determination. These new insights have generated enormous excitement because it appears that the first molecular counting mechanism has been discovered and its discovery relates closely to both aging and cancer.

For those who may be unfamiliar with this field I will give a brief introduction (for reviews, see Hayflick, 1980a, 1984; Norwood *et al*., 1990).

This story has its historical roots in the discovery of cell culture techniques at the turn of this century. From the early 1900s until 1960, it was believed that cultured normal cells, given optimum environmental conditions, had an unlimited capacity to replicate and to function. Consequently, aging was thought to have little to do with intracellular events and research on aging focused on extracellular determinants.

In the early 1960s we overthrew this dogma after finding that normal cells do have a finite replicative capacity, and we interpreted this phenomenon to be aging at the cellular level (Hayflick and Moorhead, 1961; Hayflick, 1965). We pointed out that there are two classes of cells, normal mortal cells and immortal cancer cells (Hayflick, 1965). Since making this distinction, interest in the process of immortalization of normal cells has attracted an enormous amount of attention (for reviews, see Hayflick, 1980a, 1984; Norwood *et al*., 1990).

The limited ability for normal cells to replicate and function, and our observation that cryogenically preserved cells “remember” for decades at what population doubling level they were preserved (Hayflick, 1965), implied the existence of a putative counting mechanism.

## THE TELOMERE REPLICOMETER

Cell mortality and immortality are inextricably linked to aging and cancer; consequently, the importance of identifying the counter would be difficult to exaggerate.

The sought-after mechanism should not be called a clock or chronometer, because these are devices that measure the passage of time. Because the replicative limit of normal cells is only indirectly related to the passage of time but directly related to the number of DNA replications, the putative mechanism should be more properly referred to as an event counter. A device that counts events is called a meter, which would justify my suggestion that the term "replicometer" be used to designate the putative molecular event counter.

In efforts to determine the location of the replicometer, early experiments in which we fused the nuclei of old and young cultured cells to the enucleated cytoplasm of opposite-aged cytoplasts revealed that the replicometer was located in the nucleus (Wright and Hayflick, 1975; Muggleton-Harris and Hayflick, 1976).

But more progress has been made in locating and describing the replicometer in the last 10 years than was made in the previous 25 years, thanks to a remarkable confluence of observations made in several diverse biological fields (for recent reviews of this rapidly developing field, see Blackburn and Greider, 1995; Kipling, 1995; Chiu and Harley, 1997; Greider, 1998).

It had been known at least since a lecture given by Hermann Muller in 1938 (Muller, 1962) and Barbara McClintock's work in 1941 (McClintock, 1941) that the tips of chromosomes contain discrete structures called telomeres, but the precise role that these structures played in cell replication was unclear. There was some evidence that telomeres prevented chromosomes from fusing to each other end to end, and that they permitted the attachment of chromosome ends to the nuclear envelope.

In the early 1970s it was observed that the properties of DNA polymerase prevent it from fully replicating the linear ends of DNA (Olovnikov, 1971, 1973, 1996; Watson, 1972). This has been called the "end-replication problem." The problem is the inability of DNA polymerase to completely replicate the 3′ end of linear duplex DNA.

In 1970, Alexey Olovnikov, who had just heard a lecture in which my work was discussed, wondered, as he entered a Moscow subway station how normal cells might have a limited capacity to replicate (Olovnikov, 1996). When the train stopped at the station he had a remarkable flash of insight. Olovnikov saw an analogy between the train, which represented the DNA polymerase, and the track, which represented the DNA. If the train engine was imagined to replicate the DNA track, the first segment of DNA would not be replicated because it was underneath the engine at the start. This was analogous to the "end-replication problem." Olovnikov realized that this repeated shortening of the DNA molecule at each round of DNA replication would shorten the DNA molecule and might be the explanation for my finding that normal cells can only replicate a specific number of times (Olovnikov, 1996).

Because the loss of DNA that contained vital genetic information at each division seemed unlikely, Olovnikov reasoned that the telomeres might consist of some repeated nucleotide sequences that did not contain any genetic information but behaved much like a buffer. At each round of DNA replication the buffer would simply lose what portion of the DNA molecule was not copied (the telomeric ends) and thus protect the downstream genes. The length of the buffer would thus determine the number of rounds possible for DNA replication.

Olovnikov's imaginative solution to the "end-replication problem," although published in both Russian (Olovnikov, 1971), and English (Olovnikov, 1973), languished in the literature

until several discoveries, commencing in the late 1970s, began to support his armchair speculations, and in the past decade proved them substantially to be correct.

In July 1972, at the Ninth International Congress of Gerontology in Kiev, U.S.S.R., Olovnikov sought me out, and we discussed his theory. Although it sounded plausible to me, it made no lasting impression because there was no laboratory data to support his speculation, and our conversation ended suddenly when I received a mysterious telegram that involved Zhores Medvedev who was the first to note that repetitive copies of functional genes might govern, or trigger, the aging process (Medvedev, 1972).

I had met Medvedev in Moscow in 1965, and we had a continuing correspondence. We met on the day before the conference opened and parted with the promise to meet again the following morning at the first scientific sessions. He did not appear, and we feared for his safety.

Medvedev had been a strong critic of the Soviet bureaucracy since he published his first book in the West in 1969 titled, "The Rise and Fall of T.D. Lysenko" (Medvedev, 1969). In this book he castigated the charlatan, Trofim Lysenko, and his domination of Soviet genetics and agriculture with Lamarckism—the inheritance of acquired characteristics. Lysenko dismissed outright the bourgeois Morgan-Mendelian genetics and, in so doing, set back Soviet genetics for at least 20 years. Because of this book and other books published in the West, all critical of the Soviet Union, Medvedev was an irritant to the leadership of the Soviet Union, and became the first of many Russian scientists to be falsely declared insane and incarcerated in a mental institution. Efforts by many of us in the West ultimately resulted in his release. He was officially banished from attending the Kiev conference but did so anyway by taking vacation time and traveling at his own expense to see me and other Western scientists. The telegram that I received while talking to Olovnikov read, "Regret to inform you that I have not been able to use your kind invitation because some earlier arrangements with Professor Kidnapper," signed "Zhores." Medvedev correctly surmised that the internal state telegraph staff would not understand English.

I quickly ended my conversation with Olovnikov in order to help rally support for Medvedev and to determine whether he was safe. We later learned that, on instructions of the KGB, he was kidnaped by the local police and given a one-way ticket to Moscow, where I saw him at the end of my trip. (For a full description of these events see Shock, 1988.)

## TELOMERES IN HUMAN CELLS

In 1978, Elizabeth Blackburn, working with the ciliated protozoan, *Tetrahymena*, found that the telomeres consisted of a simple sequence of hexameric repeats of the nucleotides TTGGGG (Blackburn and Gall, 1978). It was later found that the telomere repeat sequence in human cells was TTAGGG (Moyzis *et al*., 1988). Like other eukaryotic organisms, the telomeres in human cells consist of thousands of repeats of the sequence TTAGGG. It is now known that this sequence is highly conserved, and is identical from the slime mold to humans (Henderson, 1995).

Many unicellular organisms and viruses have evolved a special mechanism to circumvent this problem. In these organisms, the chromosomes are circular, or the genome produces circular replicative intermediates that simply lack ends so that the problem encountered in linear chromosomes does not exist.

Calvin Harley, who had worked for several years with my system of senescent human cells, and who had a fortuitous discussion with Carol Greider, followed up on a suggestion by Howard Cooke (Cooke and Smith, 1986), and both decided to explore the possibility that the limited

proliferative capacity of cultured normal cells might be explained by diminishing telomere length. They found that the mean telomere length decreased by two to three kilobase pairs (kbp) during the entire in vitro lifetime of several strains of cultured normal human diploid fibroblasts (Harley *et al*., 1990). Later they reported that the decrease was progressive, and averaged 50 base pairs for each population doubling (Levy *et al*., 1992). The telomere shortening seen in aging normal human fibroblasts also occurs in many other normal cultured cell types. It is not an artifact of cultured cells because it is also manifest in vivo in skin epidermal cells (Lindsey *et al*., 1991), peripheral blood leukocytes, colon mucosa epithelia (Allsopp *et al*., 1992), and many other normal cell types.

Allsopp *et al*. (1992) reported that after analyzing the cultured normal fibroblasts from 31 human donors, aged 0 to 93 years, a striking correlation, valid over the entire age range, was found between replicative capacity and initial telomere length. Thus, cell strains with shorter telomeres underwent significantly fewer doublings than those with longer telomeres. The authors suggested that telomere length is a biomarker of somatic cell aging in humans, and that this is consistent with a causal role for telomere loss in aging. They also reported that fibroblasts from Hutchinson-Gilford progeria donors had short telomeres, consistent with their reduced division potential in vitro. Telomeres from sperm DNA did not decrease with donor age, suggesting that a mechanism for maintaining telomere length may be active in the germ line.

In a report by Vaziri *et al*. (1993), studies are described in which accelerated telomere loss is associated with the premature immunosenescence of lymphocytes in individuals with Down syndrome (DS), and telomeric DNA is also lost during aging of lymphocytes in vitro. Genomic DNA was isolated from peripheral blood lymphocytes of 140 individuals ranging in age from 0 to 107 years, including 21 DS patients. The DS patients showed a significantly higher rate of telomere loss with donor age (133 6 15 bp/year) compared with age-matched controls (41 6 7.7 bp/year), suggesting that telomere loss may be a biomarker for premature immunosenescence in DS patients, and that it may play a role in this process.

Telomere loss during aging in vitro was calculated for lymphocytes from four normal individuals grown in culture for 10–30 population doublings. The rate of telomere loss was approximately 120 bp/population doubling comparable to that seen in other somatic cells. Also, telomere lengths of lymphocytes from centenarians and from older DS patients were similar to those of senescent lymphocytes in culture, which suggests that replicative senescence could partially account for aging of the immune system in DS patients and in elderly individuals.

Telomeric shortening, which occurs in several classes of dividing normal somatic cells, may be the replicometer that determines the number of times that a normal cell is able to divide. Once a critical or threshold number of telomeric $(TTAGGG)_n$ repeats is reached, cells are then unable to divide. An alternative explanation of how telomere shortening acts as a biological clock has been offered by Wright and Shay (1992). Their telomere positional effect explanation of cell senescence is based on a novel two-stage model.

## ACHIEVING IMMORTALITY

The essential remaining question in this fascinating story is this: How does that class of cells that we identified as immortal (Hayflick, 1965) avoid telomere shortening that, if it occurs, would lead to their demise?

The answer to this critical question originated in studies with *Tetrahymena* by Greider and Blackburn (1985), who discovered the ribonucleoprotein enzyme terminal transferase called telomerase. They found that telomeres are synthesized de novo by telomerase, a ribonucleo-

protein enzyme that extends the 3′ end of telomeres and thus elongates them. This ribonucleoprotein complex contains a reverse transcriptase and RNA template for the synthesis of the repeated sequence (Shippen-Lentz and Blackburn, 1990). It was simultaneously reported that cancer cells have shorter telomeres than do adjacent normal cells (Hastie *et al*., 1990; de Lange *et al*., 1990), thus providing the first link for the role of telomeres in cancer biology.

Telomerase was later found to occur in extracts of immortal human cell lines (Morin, 1989; Counter *et al*., 1992) and in about 90% of all human tumors studied (Chiu and Harley, 1997). The telomerase RNA component was cloned a few years ago (Feng *et al*., 1995), and subsequently, the catalytic portion of the enzyme was cloned (Nakamura *et al*., 1997). This enzyme is the only known reverse transcriptase that is necessary for normal cell activity.

Unlike normal mortal cultured cell strains, immortal cultured abnormal cell lines, produce telomerase. Thus, the telomeres of immortal cells do not shorten with serial passage in vitro (Harley *et al*., 1990).

In recent years telomerase has also been found to be expressed in some classes of normal cells. These include fetal tissue, normal bone marrow stem cells, testes, peripheral blood lymphocytes, skin epidermis, and intestinal crypt cells (for references, see Chiu and Harley, 1997). These cell lineages have high turnover rates or are in a continuously replicating pool of differentiating cells. It is important to note that the level of telomerase activity found in these normal cell populations is significantly less per cell than that found in cancer cell populations (Chiu and Harley, 1997).

The observation that telomeres shorten as normal cells divide, provides the first evidence for the putative replicometer (Harley *et al*., 1990). This, in combination with the discovery of the enzyme telomerase (Greider and Blackburn, 1985), has gone very far in explaining why most normal somatic cells have a finite capacity to replicate in vivo and in vitro, and how immortal cancer cells might circumvent this inevitability.

Early this year it was reported that normal, mortal human cell strains could be immortalized with retention of their normal properties by transfecting them with vectors encoding the human telomerase catalytic subunit (Bodnar *et al*., 1998). Thus, the normal longevity determination mechanism of telomere shortening in normal human cells has been circumvented. This has provided direct evidence proving the role of telomere shortening in cell senescence and telomerase expression in cell immortality.

This discovery has profound theoretical and practical implications that include the immortalization of highly differentiated normal human cell types for the production of commercially important proteins.

Because exquisitely sensitive methods exist for the detection of telomerase in a single cell, this procedure will likely be exploited as a sensitive diagnostic tool to detect the presence of cancer cells in clinical specimens. About 90% of all human cancers reveal telomerase activity (Shay, 1998). Other researchers are exploring the possibility that telomerase inhibitors might be found that could be used therapeutically in the treatment of cancer.

## TELOMERES AS LONGEVITY DETERMINATORS

I would like to suggest an alternative hypothesis for the role of telomeres in aging. I propose that telomere shortening may be the molecular equivalent of longevity determination that I defined earlier. The age changes that occur in normal cultured cells have been reported over the past 35 years, and number in the hundreds (Hayflick, 1980b). These changes represent increasing molecular disorder, or aging, and all compromise the internal milieu ultimately leading to

loss of cell function. Thus, the number of population doublings that a normal cell is capable of undergoing may be the in vitro expression of maximum potential longevity that is never reached in vivo. The limit is never reached in vivo because the hundreds of molecular disorders that herald the approaching loss of replicative capacity increase vulnerability to pathology and then to organismal death well before diminished telomere length stops DNA replication.

## WHAT SHOULD BE THE GOAL OF RESEARCH IN BIOGERONTOLOGY?

Biogerontology is a unique field because, unlike other fields of biology, I do not believe that understanding the aging process to the extent that humans will have the power to manipulate it, is a desirable goal. In fact, I cannot think of any scenario that would benefit individual humans or society in general by our possessing the power to arrest or slow the fundamental processes of aging.

Consider the most benign means of manipulation—a sugar-coated pill that, consumed daily, would result in slowing the aging process or even arresting it. The rich and powerful would probably have first access, and I would be uncomfortable with this undemocratic means of distribution, to say nothing of favoring these particular classes of people over others. Given equitable opportunities for distribution, it is likely that many people, leading unhappy lives, would choose to forego tampering with their aging processes. Many bizarre situations would occur if some chose to be treated and others not. I can visualize, for example, parents opting to take the pill and children not, resulting in children becoming biologically older than their parents.

An essential question that remains unanswerable even today is when would be the best time to arrest one's aging processes. Many people in their 60s and 70s believe that those ages are the best times in their lives. If they are in good health, then retirement also can bring freedom from parental responsibilities, time to do as one pleases, and even with modest economic resources life satisfaction can be at its peak in these older ages. Arresting the aging process at a younger age would result in the loss of this opportunity. Many who lead unhappy lives will not chose to live longer. The asynchronies in biological ages of family members, friends, and associates that would result from the ability to tamper with the aging process would lead to horrendous impacts on virtually every human institution. And, what would be done with the tyrants, serial killers, and a host of other undesirables who might want to live longer or even forever? As for me, I would prefer to pay the price of my own aging and ultimate death in order to ensure that these processes continue to be universally experienced.

## SOME FINAL THOUGHTS

It is only within the past few years that the field of biogerontology has emerged as a legitimate area for scientific inquiry. Forty years ago, when I first realized that our work might be telling us something about aging, only a few dozen intrepid people did research in gerontology. At that time the stigma of working in a field recognized for centuries as a black art commanded little scientific respect.

And, those using cell cultures in gerontological research 40 years ago were doubly damned because cell culture itself was just emerging then from condemnation as a black art.

Today, biogerontology is enjoying a level of interest that 40 years ago could not have been imagined. At that time to admit an interest in the biology of aging was to commit professional suicide. The field was simply not well regarded, and certainly not in the mainstream of biological sciences. Today, biogerontology is an enormous enterprise. Young scientists are

encouraged with fellowships to labor in the field and, for the first time in the history of modern biology, you can expect to have a successful career studying the biology of aging.

Although today biogerontology is flourishing, it still has far to go before it emerges completely from what has been analogous to alchemy in the middle ages, where the main goal was to turn base metals into gold. The popular belief by many lay people (and some gerontologists) that the goal of research in gerontology is to make us all immortal is equivalent to the belief that the goal of modern chemistry is, like ancient alchemy, the transmutation of base metals into gold.

I do not believe that our goal should be to tamper with the fundamental processes of aging, even if that were possible. The problems that would be created far outweigh any potential benefits (Hayflick, 1996). Indeed, I cannot imagine any scenario where tampering with the aging process would be beneficial.

Biogerontologists have an obligation to determine the goal of this profession because the glib belief that it should be to arrest or stop the aging processes will not accrue to our benefit or that of society.

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