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A Survey of Aging Research

1. Abstract

The spin-off from the Human Genome Project is resulting in a rapidly escalating base of knowledge about life processes at their most fundamental level. Knowledge gleaned from that endeavor may offer the prospect of slowing the aging process. Dr. Francis Collins, Director of the National Human Genome Research Institute, has predicted, "By 2030, major genes responsible for the aging process in humans will likely have been identified, and clinical trials with drugs to retard the process may well be getting underway." A growing number of scientists recognize extension of the maximum life span as a possibility.

Our profession cannot lay claim to expertise in the area of mortality while ignoring important scientific research into the causes of aging. Otherwise, like generals preparing for the last war, we will ignore events that could destroy the assumptions underlying our projections of the future. This paper provides the actuary with a brief overview of the subject, along with references for those interested in conducting their own review.

2. Introduction

We are in the midst of a revolution in biological knowledge, one that will ultimately give us control of life at its deepest levels. This research is, arguably, the most important endeavor taking place on the planet today, and the technologies being developed are the most stunning since the discovery of fire.

Although research into the aging process was begun long before the Human Genome Project, it has benefited greatly from the powerful tools and techniques spun off from that endeavor. This paper surveys current research, reviews what has been learned so far, and looks at future possibilities. After a brief historical perspective, it addresses issues such as:

- What is aging, and why does it occur?
- Do all organisms age?
- Is there an evolutionary basis for aging?
- What are the main theories of aging?
- What are telomeres, and what role do they play in replicative senescence?
- Why is oxidation implicated in the aging process?
- What are mitochondria and how do they affect aging?
- What is glycosylation and how does it affect aging?
- Does caloric restriction retard aging?
- Which genes are involved in aging? Can we intervene in their functioning?
- What impact would a significantly extended human life span have on the world?

3. Perspective

3.1. Current State of Research

Aging research today is about where electronics was shortly after the invention of the transistor: its potential is yet to be realized. Scientists are in the position of the fabled three blind men encountering the elephant, each touching and describing different parts and coming to different conclusions. As is typical of any developing field, there is often no consensus on the phenomena under investigation, the interpretation of data, or the causes behind the phenomena. Discovery of one more sign of aging is often confused with discovery of the cause of aging. Theories abound, and each investigator champions his own as the most likely explanation. In 1990 the Russian geneticist and biogerontologist Zhores Medvedev listed and categorized over 300 theories of aging.

At this point, there is not even agreement as to what aging is or how to define it. At a very general level, we can describe aging as a loss of function with time. Past that, we cannot really provide a very good description. That is, essentially, what current research efforts are aimed at discovering. The focus of much of that effort has been to learn what is happening at a cellular, genetic, and molecular level. Until we do so, scientists maintain, we do not really know what aging is.

3.2. Past Efforts, Funding

As one might imagine, past efforts were hampered by considerable prejudice. Funding was not as readily available as for other fields, and what was available was directed more toward standard gerontological research than true aging research. The general mindset held it wasn't possible to affect the aging process, and, therefore, attempts to do so were a waste of money. Attitudes such as this are disappearing, however, as the base of solid research grows. And while many scientists, including those in the field of aging research, remain skeptical and question deeply the proposition that we might eventually slow the aging process, a growing number are voicing the opinion that it is a goal we will eventually attain.

3.3. Caveats

Eternal youth has been a dream of mankind for as long as we know. It is not surprising, then, to discover that a multi-billion dollar industry has developed, with the help of pseudo-science and a news media that capitalizes on sensationalism, to prey on those wishing to believe in such dreams. The books and articles referenced at the end of this paper should provide a needed antidote for much of that. Scientists such as Jay Olshansky and Bruce Carnes say, "The bad news is that these new messages contain the same false promises that have been marketed and sold for thousands of years. The good news is that falsehoods, deceptions, and exaggerations are unnecessary. Scientists are on the threshold of discoveries about aging that are likely to have consequences for personal health and longevity that we could only have dreamed of just a few decades ago. We are optimistic that aspects of the aging process will eventually fall within the control of the biomedical sciences – permitting humanity, for the first time, an opportunity to influence the biological forces that govern life and death."

It should be noted that in a short paper such as this, it is not possible to cover even the main concepts concerning aging. Many interesting ideas must either be put aside entirely or covered only briefly. And a host of details must be passed over, which is unfortunate for at least two reasons. First, the details are often crucial to a complete and informed understanding.

Second, it does a disservice to both author and reader by forcing oversimplification. In order to acquire a balanced perspective, then, the reader must conduct deeper inquiries on his own.

4. What is aging?

4.1. Measuring Aging

What is aging? And how can we measure it? Chronological age is clearly not the same as biological age. Even within the same species, some organisms age more rapidly than others. However, we do not currently have a good set of biological markers for gauging an organism's "true" age, although this is changing. We might suggest "cell age" as a proxy for true age. But most of the cells in the human body are replaced every five to ten years. And while some cells, such as skin and intestinal cells, divide often, others, such as heart and nerve tissue, seldom, if ever, do. Furthermore, should a cell born of a recent cell division have its age measured from the point of division, or should its age be measured from inception of the fertilized egg? Or some point in between? These are difficult questions that required a more refined knowledge of cellular processes.

4.2. Cellular Aging

It can be argued that the beginning of modern aging research can be traced to the 1961 paper published by Leonard Hayflick and Paul Moorhead. In that paper, the two scientists established for the first time that, *in vitro*, normal human cells are able to divide only about 40-60 times. This later became known as the "Hayflick limit", and the quiescent state of the cells as "replicative senescence". It has recently been discovered that the cause of this phenomenon can be attributed to the telomere, which is discussed later.

Before the Hayflick / Moorhead paper, the prevailing scientific dogma was that cells in culture could divide indefinitely. This idea dominated scientific thought for nearly half a century, and was so thoroughly ingrained that the two scientists risked their reputations by suggesting otherwise. The great difficulty they experienced in finding a journal to publish their paper was eventually rewarded when it became one of the hundred most cited papers in the 1960s, out of some two million published.

The idea that cells in culture did not age was the result of work done by a well-respected scientist named Alexis Carrel, who was awarded a Nobel prize for other work. Carrel performed an experiment in which chicken heart cells were grown in culture for over thirty years. Each time the culture grew to its limit, a small piece was transplanted to a new dish. The cells appeared "immortal", in that they were able to divide indefinitely. Consequently, it was thought that aging in the body must result from events outside the cell. Later information indicated Carrel's methodology probably resulted in accidental contamination of the new cultures with embryonic chicken cells, which accounted for their seeming ability to divide indefinitely.

Meanwhile, the Hayflick / Moorhead work launched the field of cytogerontology, or the study of cellular aging. Since then, work by other talented men and women has continued to expand our knowledge of aging, and the tools of modern biology have grown far more powerful. While our understanding of cellular activity is far from complete, it is now understood at a level of detail that can only be described as breathtaking. And scientific developments currently underway promise knowledge at an even more refined level.

4.3. Distinctions

Several important distinctions should be made before continuing. First, understanding why we age is different from understanding how we age, and that, in turn, is different from understanding what causes us to age. Evolutionary theory, as we shall see, explains why we age quite nicely. And gerontological medicine describes, in great detail, how we age – that is, the changes that take place to our bodies with time. What causes us to age is the focus of current investigations into the aging process. Specifically, those efforts attempt to explain the cellular, genetic, and molecular events that result in the gradual decline of function we call aging.

4.4. Aging is not a disease.

Aging is not a disease, nor are the diseases of old age part of the normal aging process. Rather, they are a result of aging. They are a consequence of the body's reduced capacity to fend them off. To date, much scientific time, effort, and money has been invested studying these diseases in an attempt to ward them off. Yet, relatively little has been spent studying the aging process per se, the ultimate cause of those diseases. It would be an irony of Shakespearean proportion if research into the causes of aging were to succeed first and, by virtue of that success, result in cures for the diseases, or an understanding of how to prevent them.

4.5. Aging or Longevity?

Another crucial distinction to make is the difference between aging and longevity. Hayflick points out that there are no genes for aging. Aging is a stochastic process of deterioration that occurs after reproductive maturity. As such, it is not a phenomenon that could be selected by evolution. Aging results from a degradation of cellular processes that has multiple etiologies, such as reactive oxygen species, glycosylation, etc.

Longevity, on the other hand, depends on both genetic and environmental influences. Hayflick argues that the reason we live as long as we do is because physiological reserves, or redundant capacities, were evolutionarily selected because they enhanced the probability of survival to reproduce. These excess capacities only incidentally permit us to live for some time past that. In other words, longevity is a secondary by-product of the selection process. The price to be paid for this over-engineering is the expenditure of more energy in development.

Although Hayflick's distinction is an important one to understand in any discussion of aging, the concepts of "aging" and "longevity" will be used interchangeably in this paper. The proteins that stave off or repair the effects of those degradative processes, and the behavioral and environmental factors that minimize them, all have the ultimate effect of slowing the aging process. So, aging and longevity determination will be spoken of as if the two processes were the same, though in fact they are not.

5. Do all organisms age?

Bacteria and many other single-celled organisms don't age. That doesn't mean they don't die, just that they are capable of dividing indefinitely. They still may be eaten, starve to death, or succumb to environmental factors. Some animal species have very long chronological life spans, while others have very short ones, and some do not seem to age at all. Animals that reach a fixed size in adulthood, age. That includes mammals and birds. But some animals, mostly the more primitive species, do not reach a fixed size and do not age. Examples are some lobsters, reptiles, and many fish. Non-aging animals simply grow larger, without experiencing a decline in physical capability or reproductive function. Again, that doesn't mean they don't die. There are any number things that can happen to them. They just don't age. It may seem strange

that the later, more highly evolved species would experience aging while more primitive species, such as sharks, have no fixed life span.

Caleb Finch, a neurobiologist at the University of Southern California, has been described as one of the most knowledgeable and deepest thinkers about aging. Finch and co-author Robert Ricklefs, Professor of Biology at the University of Pennsylvania, say, "The variety of aging patterns in mammals and plants has prompted many biologists to ask whether senescence can be modified by evolution."

It has been noted since antiquity that large animals seem to age more slowly than small animals. Allometric equations are used to quantify the relationships between life span and body size / brain size / metabolic intensity / etc. The general idea is that if a relationship can be found between part of an animal and the whole animal, it may provide some general insight into the aging process. Such relationships are correlational, of course, not causal, and there is no explanation or mechanism offered for why the relationship exists.

Generally speaking, animals with large body sizes, low body temperatures, low blood sugar levels, and low oxygen consumption rates live longer. That's what makes some species of birds such an enigma. Their metabolic rate is high enough to maintain body temperatures some 3°C higher than humans. (For every 10°C rise in temperature the rate at which chemical reactions take place in animals increases by a factor of two to three times.) Birds also have blood sugar levels that would be considered dangerously diabetic in humans, some 2-10 times higher. And they consume 5 times as much oxygen. Yet, some species live up to 3 times longer than would be predicted on the basis of allometric relationships alone.

Birds appear to use their energy resources in a far less damaging manner than many other animals. It is speculated that the rigors of flight are so demanding they must stay at a near-optimal physiological condition. If so, birds may have experienced strong selective pressures to evolve genes that keep the cells of their bodies in finely-tuned working order. Their cellular maintenance and repair systems may be superior to that of other animals, in which case we may be able to learn much about the aging process from them.

6. Is there an evolutionary basis for aging?

6.1. The Origins of Aging

To risk a pun, aging is quite old. It appears to have made its entrance at about the same time sex did, hundreds of millions of years ago. That's when eukaryotic cells (ones that have a nucleus) evolved from prokaryotes (which do not). One trend in evolution was for the eukaryotes, such as Paramecia, to become larger, because size imparts certain advantages even in the microscopic world. But past a certain point, a cell has difficulty manufacturing all the proteins it needs. In Paramecia, the amount of DNA available for protein manufacture was expanded to meet those needs by replicating it many times. This "operational DNA" was sequestered in a separate macronucleus within the cell. The DNA required for reproduction was housed in a smaller micronucleus.

Paramecia can only divide about 200 times before they become replicatively senescent, that is, unable to divide anymore. Presumably, this is due at least in part to damage to the operational DNA in the macronucleus. But if paramecia conjugate, that is, have sex to exchange micronucleus DNA, the cell is rejuvenated and goes on dividing. The macronucleus is destroyed in the process and created anew from the revitalized micronucleus.

When life became multicellular, the partitioning of DNA was carried a step further. It was housed in specific germ cells, which were separated from the somatic, or non-reproductive cells. But the somatic cells of multicellular organisms have the same problems the early Paramecia macronucleus had. They age, and as a result we eventually die. Ricklefs and Finch comment, "It is said that the germ line is potentially immortal because it persists between generations, whereas the soma is mortal because its cells die with the individual."

6.2. Lineage Selection

The concept of lineage selection explains some of the above observations. In a line of cells, it is thought that the manner in which the clones avoid eventual genetic deterioration is through the selective elimination of defective lineages. For it to work, daughter cells must be produced at a higher rate than the rate of defects. Also, new cells must be produced constantly in order to take the place of lineages that have been eliminated. This is where animals that grow to a certain size and then stop growing run into trouble, as do tissues such as heart or nerve cells. Ricklefs and Finch explain, "… when the accumulation of genetic defects, or other kinds of damage, in somatic cell lineages is the underlying cause of aging, and when damaged cells are not replaced by continuous cell replication, then aging and eventually somatic death become inevitable." They also say, "… the potential immortality of the germ line does not seem to be tied up with sexual reproduction after all. Rather, it appears to be achieved by cells proliferating rapidly enough to replace those that have by chance deteriorated genetically. The soma itself cannot employ this strategy to any great extent because it either cannot afford or cannot manage the degree of cell turnover needed to continually rejuvenate the clone."

6.3. Haldane / Medawar

Why wouldn't a trait as harmful as aging be eliminated by evolution? J.B.S. Haldane, one of the twentieth century's most talented biologists, made an observation about Huntington's disease that was to have an impact on aging theory. Huntington's disease, which strikes in middle age, has received much attention from geneticists over the last several years. Those afflicted with the disease suffer progressive neurological deterioration which results in loss of control over mind and body during the decade or two before they die. Haldane pointed out that the reason Huntington's disease is so common is that it doesn't appear until midlife, after the years of sexual reproduction.

Sir Peter Medawar, the brilliant British immunologist and Nobel prize winner, immediately grasped the implications of that idea and formulated a concept in mathematical terms that is similar to one that actuaries are familiar with: *The force of natural selection declines with age.* The consequences of Medawar's theory explain biological observations that would be difficult to understand otherwise. Steven Austad, Professor of Zoology at the University of Idaho and a leading expert on the subject of aging, says, "His theory neatly resolves the paradox of aging and explains all the patterns of aging that we have seen to date."

Ricklefs and Finch say, "Evolutionary theories of aging have helped biologists to resolve the dilemma of how a population can maintain a seemingly harmful trait in the face of natural selection." Waning natural selection leads to gradually increasing physical decay by two routes. The first is that populations sometimes accumulate deleterious mutations whose cumulative side effects cause dysfunction in later life, after the reproductive years. The second is that antagonistically pleitropic genes can be maintained in a population despite their harmful effects.

The concept of antagonistic pleitropy was proposed by George Williams, an evolutionary biologist at the State University of New York. A gene that expresses itself in more than one

attribute of an individual is called pleiotropic. An example of a pleitropic gene is the one responsible for myotonic dystrophy, which also causes cataracts, diabetes, irregular heat beat, malfunctions of the gonads, and mental retardation. That is, it causes a variety of effects in a wide range of organs. Sometimes the effects of such a gene may have opposite effects on an organism's evolutionary fitness. For example, a group of genes that gives a heightened immune response in a chicken may also require a greater expenditure of energy. Greater immune capacity obviously helps survival. But during breeding, which requires greater energy resources for fighting and reproduction, the additional biological "drag" may hinder survival or the ability to leave offspring. As another example, a gene that hastens the hardening of bone in early life, thereby adding strength to the organism, might produce arterial calcification later, resulting in arteriosclerosis.

John Medina, a molecular biologist at the University of Washington School of Medicine, comments, "The idea that natural selection doesn't care one whit about aging has most profound consequences for its evolutionary context. … This means that aging is an evolutionary landfill of potentially confused genetic processes, subject as much to whim as to direction."

6.4. Examples Illustrating the Evolutionary Basis of Aging

There is a corollary to Medawar's hypothesis: the effect of natural selection is dependent upon the relative hostility of the environment. A highly hazardous environment will make the effects of natural selection fade rapidly with age. Steven Austad reasoned that an animal's reproductive strategy will have a short time-horizon in such circumstances. It makes little sense to invest resources in strong immune systems and other longevity-enhancing defenses if you are likely to die in the next few weeks or months. It is better instead to reproduce quickly and copiously. Austad decided to see if Nature had already performed the experiment he envisioned.

Opossums age very rapidly, seldom living more than two years, which is a rapid rate of aging for an animal of that size. They are slow-moving and not well-armed. Consequently, they most often die from predation. Austad began searching the southeastern United States for an island that was too far for opossums to swim to, and that had been separated from the mainland for a long time. An important characteristic of islands is that they are generally too small to support predators. That meant the environment would not be "hostile". Sure enough, Sapelo Island, off the coast of Georgia had a population of opossums that averaged about 25% greater longevity.

Their litters were smaller, averaging four to six pups versus the normal six to nine. Their reproductive systems aged more slowly, too, allowing a second breeding season, rare among mainland animals. Finally, collagen in the tail tendons appeared to be aging less slowly. Collagen is a protein that produces strong, flexible, relatively elastic fibers used for structural support. When it breaks down, it is not replaced. With time, the molecules develop chemical cross-links and become less flexible. The Sapelo opossums had fewer cross-links. By all measures, Sapelo Island opossums aged more slowly, in accordance with what evolutionary theory would predict.

Another example that lends support to evolution's role in the aging process was provided by Michael Rose, an evolutionary biologist at the University of California at Irvine. Humans have engaged in artificial selection, as opposed to natural selection, for thousands of years. It is called "breeding". Rose performed an experiment on fruit flies in which he decided to slow down the rate at which natural selection waned with age by selecting only the eggs from the oldest flies in each generation. Beginning with eggs laid by 28 day old flies, he eventually wound up gathering eggs from flies as old as 70 days. He now has flies that live twice as long as

the original ones. They live healthier, longer lives, are more active, and more resistant to dessication, starvation, and stress from heat and chemicals. This experiment and other lines of evidence lead scientists to reason that the life span is plastic; that is, it is susceptible to being perturbed.

6.5. The Evolution of Life Span

Hayflick notes that the life span of different animal species has increased as they evolved and speculates that longevity must be determined by less than one percent of the total number of genes. This is still a large number of genes, however, since the human genome contains about 30,000 genes (and that number may grow). Hayflick also cites the connection between brain size and longevity, along with archaeological measurements of cranial capacity, as partial evidence for the belief that hominid life spans increased rapidly until about 100,000 years ago before stabilizing at roughly the current maximum.

7. What are the main theories of aging?

Bernard Strehler proposed a set of requirements that any successful aging theory should meet. It should be able to explain why the proposed phenomenon is deleterious, progressive, intrinsic, and universal. That is, it should explain why losses in physiological function occur, why they are gradual, why they cannot be corrected, and why they occur in all members of a species.

As previously mentioned, there are almost as many theories on aging as there are researchers. Those who are interested in inquiring further will find a captivating succession of theories and experiments that deal with aging. Evolutionary theories have already been mentioned. Another set of theories, the wear and tear group, propose that aging results from the effects of mechanical or biochemical wear and tear on the body, its organs, tissues, and cells. Neuroendocrine theories point to the effects of hormones. Hormones are specialized proteins that enable cells in one part of the body to respond to conditions in another part of the body. They are often involved in complicated feedback loops that control vital metabolic and reproductive pathways. Their effects are generally very powerful, and if they get out of balance the results can be damaging.

Even though the brain accounts for only about two percent of a person's body-weight, it consumes about one-fourth of the body's oxygen intake. Because it controls or influences almost every organ in the body, the brain has been mentioned as a candidate for the cause of aging, also. The immune system weakens as we age, and also becomes more likely to mistake 'self' for 'non-self' and attack the body it was intended to defend. Immune system theories argue that disorder in the immune system results in the pathologies of old age. Rate of living theories assume that the length of life is dependent upon how fast it is lived, and aging is due to the depletion of some vital substance. Although the original form of the theory is no longer accepted, it, and its successors, pointed to other ideas that have been incorporated into the mainstream thinking.

Over time, cells accumulate waste. If they are unable to dispose of this waste, the accumulated toxins could harm the cell. This is the central idea behind waste product accumulation theories of aging. Programmed aging theories hold, in one form or another, that aging results from changes ordered by our genetic blueprint. Errors and repairs theories ascribe aging to an accumulation of defects resulting from imperfect repair of damaged proteins and

DNA. There are many other theories, such as order to disorder theories, exercise theories, cross-linking theories, and a host of others. We shall examine a few of these in more detail now.

8. What are telomeres, and what role do they play in replicative senescence?

Within the last several years, an understanding of the cause of the Hayflick limit has been achieved. Cell division is regulated in many ways and at many different points in the cell cycle. Telomeres are one regulating mechanism. At the ends of each chromosome are structures called telomeres, which are nothing more than thousands of repetitions of the nucleotide sequence TTAGGG (thymine, adenine, guanine). The telomere is analogous to the cap on a shoestring; it keeps the DNA from unraveling. It has other functions too, such as regulation of the peritelomeric genes, but protecting the chromosome is the primary function. A biochemical quirk causes a shortening of the telomeres each time a cell divides: the cell's copying mechanism simply cannot copy the chromosome's DNA completely, which results in progressively shorter telomeres. When the telomere reaches a certain length, the cell is no longer able to divide.

So, is replicative senescence the cause of aging? At first, researchers thought this might be the case. Michael Fossel, professor of clinical medicine at Michigan State University and holder of an M.D. and a Ph.D. in neurobiology from Stanford, thinks it may play a major role. He argues that, while we cannot escape death, we can avoid aging, and even reverse it. Some cells, such as cancer cells, are able to divide indefinitely. The reason they can do so is that they are able to turn on a gene that produces telomerase, a protein that enables the cell to re-lengthen the telomeres. By doing so, cancer cells are able to go on dividing.

Researchers at Geron corporation have shown that the addition of telomerase to cell cultures will rejuvenate senescent cells and allow them to divide again. This may presage the ability to reinvigorate some types of aged tissues, although there are concerns that continued cell division could lead to cancer. The researchers have also found telomerase inhibitors that may be useful in treating cancer. Most cancer cells have divided so many times their telomeres are extremely short. It is only their ability to produce telomerase that enables them to keep dividing. By inhibiting the production of telomerase, it may be possible to prevent continued division.

Fossel argues forcefully and in detail that telomeres and telomerase are not only the key to slowing aging, but the path to reversing it. Few researchers would agree with his position, however. As mentioned, many cells don't divide, yet still age. While admitting that telomeres clearly play a role in some aging processes, most researchers do not think they are the cause of aging.

9. Why is oxidation implicated in the aging process?

The observation that lower body temperatures go hand in hand with slower aging led eventually to the rate of living theory. Although the original version of this theory has long since been discarded, it pointed to the idea that there may be something intrinsically damaging about energy use. A biochemist named Denham Harman proposed the free radical theory of aging, which provides a plausible mechanism for the way that aging and metabolism are related – through damage caused by the oxygen consumed in energy production.

A very strong inferential role has been made for free radicals in the aging process. The most convincing evidence comes from experiments with a group of chemicals called antioxidants. During metabolism, oxygen is progressively converted to superoxide anions, hydrogen peroxide, and hydrogen peroxide radicals, all of which are highly reactive. These

metabolic by-products combine readily with lipids, proteins, and DNA, and cause cascades of reactions that harm the cell and prevent it from performing its function properly.

The cell has devised mechanisms to protect itself from the damage these free radicals wreak, however. A host of "firefighter" molecules keep them under control. These molecules include dietary antioxidants, such as vitamins C and E, as well as antioxidants manufactured by the body, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase. Superoxide dismutase converts superoxide to hydrogen peroxide, and peroxidase and catalase convert the hydrogen peroxides to water. But these protective reactions aren't perfect. Several percent of the total oxygen processed still escapes into the cell where it does damage. Controlling that leakage appears to be one key to slowing the aging process. Researchers at The University of Texas inserted extra copies of the superoxide dismutase and catalase genes into fruit flies, resulting in an increase of 30% in both life expectancy and maximum life span.

In general, animals with higher levels of SOD have longer life spans. Humans have the highest levels and also the longest life span. It is fruitless to consume SOD, however, as some health food faddists recommend, because it is destroyed during digestion. Moreover, indiscriminant ingestion of various vitamins and antioxidants is probably not a good idea. Recent research has shown that the wrong quantities of antioxidants may actually increase the body's oxidative stress. One of the researchers at Geron is now with a company that claims to have developed a proprietary antioxidant that reduces oxidative stress by 25% compared to a standard 400 unit vitamin E tablet.

10. What are mitochondria and how do they affect aging?

Each cell of your body contains several hundred to several thousand mitochondria. These tiny power plants produce the cell's energy currency, adenosine triphosphate (ATP). Whereas, the DNA in the nucleus of a cell comes from both the mother and the father, each mitochondria has but a single set of DNA, which comes only from the mother. Scientists think mitochondria represent the vestigial remnants of an archaic infection that took place when life on the planet was still young. The cell that became infected was large and very good at gathering food, but poor at extracting energy. The parasitic bacteria was small and inefficient at gathering food, but its energy extraction pathway was excellent. Over millions of years, the two formed a symbiotic relationship, one contributing a hospitable environment with plenty of food, the other an efficient energy pathway. Gradually, most of the genes of the tiny invader were eliminated or incorporated into the genome of its host. All that remained behind was a tiny amount of DNA in the mitochondria itself. A decade ago, scientists used mitochondrial DNA and estimates of genetic drift to conclude that all people on earth descended from a common female ancestor who lived in Africa some 240,000 years ago. The press quickly dubbed her "Mitochondrial Eve." The statistics in the study were flawed, however, and a great scientific brouhaha erupted, followed by a succession of further studies, arguments, and counterarguments.

Since mitochondria are responsible for most of the energy produced by the cell, they are also subject to the greatest damage by oxidation reactions. Over 90% of the oxygen consumed by a cell is burned in the mitochondria to produce ATP. It has been estimated that 1-4% of all oxygen used by mitochondria results in reactive oxygen species (ROS) molecules. As might be expected, since mitochondria are so close to ground zero, the leakage of oxygen and free radicals wreaks havoc with them – even more so since mitochondrial DNA is not protected by proteins the way nuclear DNA is, and it doesn't have the same repair mechanisms to mend it.

When a protein is oxidized it generally becomes inactive and unable to perform its function as a structural molecule, an enzyme, or a hormone. Oxidized cell membranes become less resilient and fluid, and oxidized lipids in the blood probably contribute to many vascular diseases. One product of lipid oxidation is lipofuscin, a yellow-brown age pigment that accumulates in some cells as they age. As much as 30% of the heart cell may eventually consist of lipofuscin. Although no one knows for sure whether this harms the cell, it is thought that it represents a burden to cellular function. Lipofuscin accumulation occurs more rapidly if the diet is deficient in vitamin E.

Lipoic acid is changed to a very powerful antioxidant in the body that protects the mitochondria. Bruce Ames, of the University of California at Berkeley, fed older rats food laced with lipoic acid. Within a short period, their liver cells deflected antioxidants with more resilience and the older rats scrambled about with new spirit and a sleeker look. It should be noted that although animals fed antioxidants live longer, this does not mean they age more slowly. An increase in life expectancy is not the same thing as an increase in maximum life span.

11. What is glycosylation and how does it affect aging?

Another key factor in the aging process is blood sugar level. Some products of glucose metabolism interact with important enzymes, proteins, fats, and DNA. When your Thanksgiving turkey turns that beautiful golden-brown color in the oven, it does so courtesy of the Maillard reaction, named for the French chemist who discovered that, under heat, glucose bonds to proteins to form the familiar, caramel-colored product typical of cooked meats. Until recently, it was not understood that the same reaction can take place at body temperatures. It has been known for some time that diabetics have glucose attached to some of their hemoglobin, and also seem to undergo what resembles rapid aging. Anthony Cerami, a Rockefeller University biochemist, reasoned that aging might result, at least in part, from the Maillard reaction. He called the chemicals that are formed AGE products, which stands for Advanced Glycosylation End-products. Glycosylation of collagen molecules, which form part of the skin, can result in cross-links that lead to a loss of flexibility and elasticity. There is also evidence that free radicals can accelerate the formation of AGEs. Once proteins become glycated, they can catalyze further damage by interacting with free radicals from other sources. Glycosylation may also play a part in the formation of atherosclerotic plaques by trapping LDL cholesterol in the artery walls. Proteins that have been affected by glucose become less soluble and less likely to be broken down by enzymes designed to destroy damaged chemicals. Glycosylation of DNA can interfere with DNA repair and possibly even result in mutations.

Certain scavenger macrophages are specialized to devour “browned” proteins, and it is thought that the body must have protective mechanisms similar to the antioxidants to protect it from AGEing. In a recent study conducted by the NIH, it was determined that a drug called ALT-711 snips the bonds or crosslinks created in arteries and other tissues when glucose attaches to collagen. This, in essence, reversed the age of the blood vessels, giving them greater flexibility. Anthony Cerami and others reported in the March 14, 2000 *Proceedings of the National Academy of Sciences* that an advanced glycation end-product cross-link breaker could reverse age-related increases in myocardial stiffness in dogs.

Between oxidation and glucose metabolism, you might say we either “rust” or “caramelize”. The findings regarding the impact of oxidation and glycosylation on the cell imply that life is inherently destructive and, therefore, self-limiting. In other words, life is a

biohazard. All is not bleak, however. In the September 1, 2000 issue of *Science*, researchers reported they have discovered drugs that mimic antioxidants in the body and extend the life span of the worms they used. One of the scientists, Simon Melov, says, "This is the first robust demonstration that drugs can extend life span." They are now working with mice, and scientists in Massachusetts intend to test the drug in clinical studies of older people who have suffered stroke.

12. Does caloric restriction retard aging?

The original rate-of-living theory received support from earlier work done by Clive McKay, a Cornell University veterinary nutritionist. In 1935, McKay published a paper in which he reported he had found a way to slow aging in laboratory animals. By reducing the number of calories his rats ate by 30-40% while still ensuring they received the nutrients necessary for good health ("undernutrition without malnutrition"), he found his rats lived 30-40% longer than rats fed *ad libitum* (to satiation). Tumors were retarded, and the animals' health was improved in almost every measurable way. The frequency of cancers was reduced, and the animals lived longer, healthier lives.

Counter-arguments were made that this was a spurious result because lab rats have been bred to eat more so they will grow faster. They also get little exercise and are extremely inbred. According to this line of reasoning, lab rats live shorter lives than wild rats, and a return to the normal life span was all that was being witnessed. However, these experiments have been replicated many times with many different species with the same results, and researchers are beginning to think that caloric restriction may actually slow the aging process in some way. It is currently being tried with rhesus monkeys at the NIH primate center. One of the interesting things discovered so far is that the additional longevity does not seem to be a result of reduced metabolism. After initial swings, metabolism stabilizes at or slightly above the original rate. Other effects have also been observed. The animals mature at smaller body sizes, and their reproduction is delayed. Roy Walford, a biogerontologist at the University of California, has been one of the few people willing to undergo the rigors of a 30-40% reduction in his diet. He does not regard his effort as an experiment (sample size of one), but has been on a strict diet since 1987. (Walford was also one of the inhabitants of Biosphere 2.)

The mechanism by which caloric reduction accomplishes its work is not clear, but we are beginning to get some indications. An MIT molecular biologist named Leonard Guarente recently announced that he had identified both a yeast gene and a metabolic enzyme that help explain the anti-aging effect of caloric restriction.

Stephen Spindler, a professor at the Department of Biochemistry at the University of California at Riverside, has used micro-chip arrays to test for changes in the expression of 11,000 genes in caloric restriction experiments. The major conclusions of his study are that many effects of calorie restriction happen rapidly and show up in both young and old animals.

13. Which genes are involved in aging? Can we intervene in their functioning?

13.1. General

Experiments with yeast, nematodes (roundworms), lab rats, and other life forms have aided our understanding of the aging process. Evolution is thrifty, so many things found in simple organisms are also found in humans. However, great caution must be taken in interpreting

results obtained from these experiments. There are many differences in physiology, and the experiments can only give indications of what to look for. Much of the work remains unclearly understood, is more than likely linked to other processes, and has been researched only in petri dishes and / or lower organisms. At this stage all it represents is enticing, suggestive work. A straight-forward extrapolation to humans is not possible.

Even if genes that affect aging are identified, the problem of how to utilize that information still remains. Gene therapy is a possibility, but a difficult and remote one at this point. And producing drugs that replace the protein products of the genes comes with its own set of problems, ranging from the size of the molecules involved and how they would be delivered to how to keep the body's cellular machinery from degrading them until they have done their job.

13.2. The number of genes involved in aging

While some researchers believe there must be an enormous number of genes involved in the aging process, others think that control of the human life span may rest with a relatively small number of genes. Dogs, for instance, didn't get large (or small) one leg or one tooth at a time. All the body parts evolved more or less together. This suggests there are probably master genes that order and coordinate the activities of many others. Such may be the case with aging also. Thomas Perls, Harvard Medical School physician and acting chief of gerontology at Beth Israel Deaconess Medical Center in Boston, believes that there are only a handful involved, perhaps 10 or so. Michael Rose agrees, suggesting as few as 36, although another 200 may fine-tune the process. "People tend to underestimate how fast the aging field is moving," says Leonard Guarente. "We're uncovering the molecular basis of aging. No, we're not at the point where we can intervene in humans yet. But we have every reason to be hopeful that day will come." Steven Austad says, "Another hint that aging is not biologically inevitable is that even organisms that do age, don't start doing so immediately. For the first part of our lives, for instance, we actually improve in virtually every bodily function, be it physical coordination, cardiovascular strength, or immune-system responsiveness."

13.3. Nature and Nurture

A study of several hundred Danish twins indicated that genes may account for 20-30% of the variability in life span. But even in highly controlled laboratory environments, genetic influences seem to account for only 30-40% of the difference in the life span of mice.

Ricklefs and Finch conclude that aging is largely induced by biochemical and mechanical wear and tear, that is by both internal and external environmental factors. But while the environment clearly influences the aging process, aging is under genetic control, and differences in aging can probably be traced to differences in maintenance and repair mechanisms. They comment, "Aging itself may not be caused by our genes, but genes may nonetheless regulate its expression and rate." Regarding the prospect of prolonging human life span they do not believe the processes responsible for aging will ever be stopped because they result from the processes of life itself. However, they say, "We may, eventually, be able to manipulate some genetically determined aspects of aging and so, eventually, extend the maximum life span of our species."

13.4. Protection & Repair

DNA has many mechanisms for damage-control, but if it cannot be repaired, it can no longer produce the proteins the body requires. Mistakes can occur in the DNA as a result of many processes. The effectiveness of the cell's maintenance and repair mechanisms is critical to controlling the effects of these events. There are at least nine different genes responsible for

DNA repair. Three common errors that accumulate in aging DNA are deletions of chunks of DNA, point mutations in the individual nucleotides, and translocations of DNA from one area to another. The p53 gene is a member of a class of genes known as tumor repressor genes. If these genes become damaged, cancer is more likely. Mutations in p53 and have been implicated in a wide range of cancers. Cellular division follows a well-defined and highly regulated process, with many checkpoints that must be passed before the cycle can continue. p53 is a protein that detects damaged DNA and arrests the cell-cycle until the damage has been repaired. If it cannot be repaired, p53 induces apoptosis, or programmed cell death. Proteins that have become damaged are similarly either repaired or degraded.

13.5. Fos

The *fos* gene codes for a protein (Fos) which is a transcription factor. It is one of many genes that must be turned on in order to cause a cell to enter a growth phase. Aging cells do not make Fos, however, and as a result the other genes do not get turned on when they should and cell replication does not take place.

13.6. Heat Shock Proteins

The body is constantly responding to its environment, and, therefore, so are its genes. One group of proteins that has evolved to deal with environmental stress is referred to collectively as the "heat shock proteins", although they deal with a great deal more than just heat. They may more properly be regarded as stress proteins (or genes). These genes deal with the introduction of heavy metals, pollutants, metabolic waste products, radiation, oxidative damage, and so forth. It has been noticed that there is a dramatic reduction in some of these proteins as the cell ages. If a gene is turned off, the cell is less able to cope with the ongoing environmental insults it must face and sometimes becomes incapacitated as a result. This, in turn, stresses other parts of the system, which must now cope with its loss.

13.7. SIR2

Leonard Guarente and other colleagues have pointed to a gene called *SIR2* as being responsible for increasing life span in both yeast and nematodes, although it operates differently in the two species. *SIR2* is a gene silencer. Guarente thinks that the link between longevity and *SIR2* is a molecule called NAD, which soaks up electrons from chemical reactions throughout the body. *SIR2* needs NAD to work. He also speculates that caloric restriction may boost available supplies of NAD by slowing metabolism. The extra NAD may ensure that *SIR2* keeps its target genes from turning on at the wrong times, which could be an underlying cause of aging.

13.8. FoxM1B

Robert Costa, professor of molecular genetics at the University of Illinois at Chicago College of Medicine, and his colleagues were able to restore the regeneration of liver cells to rates of growth typical of young mice. Earlier studies showed that age-related defects in the proliferation of connective tissue cells are associated with diminished expression of a gene called *FoxM1B*. By causing increased expression of this gene, liver cells in the aged mice were able to regenerate liver tissue which had been surgically removed. Since *FoxM1B* exists throughout the body, not just in liver cells, researchers believe their discovery might one day be used to restore other tissues also.

13.9. *Chico, InR,* and the Insulin-Signaling Pathway

David Gems, of University College London, and colleagues reported that they have extended the lives of fruit flies by 50% by mutating a gene called *chico*. The gene is involved in the insulin-signaling pathway, which is already known to regulate aging in nematodes, and now appears to play a similar role in fruit flies and yeast. This suggests the pathway may help regulate the aging process throughout the animal kingdom. In another study, Marc Tatar, of Brown University, headed a team that mutated the *InR* gene in fruit flies. The flies lived 85% longer than average. *InR* is similar to the *daf-2* genes in roundworms.

13.10. Chromosome 4

Louis Kunkel of Children's Hospital in Boston and the Howard Hughes Medical Institute in Chevy Chase, Maryland, along with Thomas Perls of Beth Israel Deaconess Medical Center in Boston, found a region in human Chromosome 4 that appears to harbor a gene or genes that confer human longevity. A genome-wide comparative analysis was made of 137 sets of long-lived siblings in order to identify the region. They are still searching for the gene or genes and hope to make the identification within a year or less. It is thought that a single nucleotide polymorphism (SNP), a subtle genetic variation, may be responsible.

13.11. Nematodes

Caenorhabditis elegans is the scientific name for nematodes, or roundworms. These microscopic animals have been the subject of much genetic study. If two of its genes, *ced-3* and *ced-4*, are turned off, cell death doesn't occur. Another gene, *ced-9*, puts the brakes on cell death. The *daf-2* gene in nematodes has been called a master gene of aging because its protein controls the actions of many other genes and when it is mutated, destroying its function, the life span of the nematodes is doubled. However, this is primarily a result of entering a special larval stage called the dauer state, which is a state of suspended animation. Tinkering with related genes, *daf-12*, *daf-15*, and *daf-23* quadrupled their life spans. Another *C. elegans* gene is *age-1*. Worms with certain alleles of this gene live greatly prolonged lives, but leave fewer offspring.

13.12. Apoptosis

Apoptosis, or programmed cell death is an important process in embryonic development and in formation of the immune and nervous systems. It is also thought to have a role in the aging process. Apo-1 is a cell membrane protein. Once its ligand binds with it, a series of events begins that ends with the destruction of the cell. Other proteins that cause cell death, such as BAX and ICE, have also been isolated. *Bcl-2* is a gene that can block apoptosis from taking place. The Bcl-2 protein accomplishes this by inhibiting the formation of free radicals. Bcl-2 has different effects on different molecular programs, depending on the type of cell it is in.

13.13. Other

A host of other genes and proteins are being discovered that play some type of role in the life / death / aging process. For instance, senstatin proteins can reverse the aging process in skin cells, nerve cells in the brain, and blood vessel cells. Longevity Assurance Genes (LAG genes) have been found in yeast that double the life span. They have also been found in humans. A gene called *Methuselah* has been found that increases the life span of fruit flies. Apparently it has an effect on stress responses. Another gene, called *Indy*, which is an acronym for "I'm not dead yet", is a metabolism-related gene found in fruit flies. Mutations in that gene can double the flies' life span. It is thought that the effects on metabolism mimic those of caloric restriction.

13.14. Progerias

The progerias are diseases that appear to mimic accelerated aging. Hutchinson-Guilford syndrome, is a disease that strikes very young children. Most of its victims are dead by the mid-teens. It is very rare because the young ages at death prevent it from being passed to another generation. The cause is a mutation in a single gene. Werner's syndrome is twice as prevalent as progeria and manifests itself during adolescence. It is the result of a recessive gene inherited from both parents. People with Werner's syndrome usually die in their mid-forties. Recently, Werner's syndrome has been linked to a defect in the gene coding for helicase, a protein that helps DNA unwind for replication.

Some scientists believe that progeria could provide genetic clues to the causes of aging. Others believe progeria is not true aging, but only mimics some aspects of aging. It is doubtful, in the estimation of these researchers, that either of these diseases will shed light on the aging process because many bodily infirmities result in deleterious changes that compromise survival but have little to do with aging. The counterargument is that the reason progeria does not seem quite like true aging is that the substrate being operated upon is the body of a very young child, which has not had time to accumulate the environmental damage that would make it appear to be more like true aging.

14. What impact would a significantly extended human life span have on the world?

14.1. Can we stop aging?

Today, we routinely create transgenic species, species whose genomes contain foreign DNA – *artificial* species that never existed before, and did not arrive via Darwin's evolutionary process. We insert and delete genes at will, and have learned to tweak the molecular switches that turn them on and off. Pharmaceutical companies insert genes for desirable drugs into livestock and harvest what the animals manufacture from their milk, urine, or blood, a process known as "pharming". Scientists grow new blood vessels from a few cells in a test tube. Others search for the genes that determine intelligence. Researchers from around the world collaborate to grow an entire human heart by 2008. Sheep are cloned from the fully differentiated tissue of an adult animal. Cattle and mice are cloned, too, and clones of clones are made. Case Western Reserve researchers, in collaboration with scientists at Athersys, have created the first functioning, replicating human artificial chromosome. Scientists at TIGR (The Institute for Genome Research) are working to determine the minimum set of genes necessary to sustain life, a feat which, if successful, will provide a de facto definition for one of mankind's oldest questions: "What is life?" Once achieved, they propose a still more audacious endeavor – to use those genes to create life. So, the very real possibility that the human life span may be susceptible to being extended, perhaps even dramatically, should not be dismissed out of hand. Our powers are growing swiftly, indeed.

But what is the real bottom line to all this? Can we stop aging? The honest answer is, "Right now, we don't know." Things are still at a rudimentary stage with respect to understanding the aging process, much less arresting it. It may well be that, even if we find ways to dramatically reduce death rates from the major causes of death – thereby extending life expectancy – we still may not be able to affect the maximum length of time people are able to live. But scientific knowledge is increasing at a break-neck pace. And even without an advance such as extending the life span, it is clear that 21st century medicine will offer radically different

opportunities for intervention into disease processes. An increase in life expectancy seems inevitable. And an increase in the maximum life span can no longer be dismissed as science fiction or wishful thinking. We seem to have reached an intellectual critical mass during the last century, and it will be interesting to see where this explosion of knowledge takes us.

Currently, there are many pharmaceutical and biotech companies working on some aspect of aging, and a constant stream of discoveries is coming out of the universities. While there is a disagreement among scientists as to whether it will be possible to slow the aging process, I believe deeply it is something we will do. The reason that "knowledge is power" is because it leads to control. Once we understand how something works, we immediately begin figuring out how to intervene in the process, so that we may control it to our own ends. We are still in our infancy with these new technologies, but they hold enormous promise.

14.2. Life and Death Over the Centuries

Hayflick offers a number of interesting statistics in his book, How And Why We Age. In 1900, 75% of the population died before age 65. Today, 70% die after age 65. Almost one-half of the babies born today will survive to age 75, or about two-thirds of the maximum life span of 122 years (set by the French woman Jeanne Louise Calment). At the beginning of this century, the leading causes of death were tuberculosis, pneumonia, and diarrhea, in the form of cholera. Today the leading causes are heart disease, and cancer. In the last century alone, life expectancy increased by more than 50%. Life expectancy in 1900 was 49, as compared to today's 75 years. A century prior to that it was about 36; during Medieval times it was 33; and during Rome's reign, it was about 22. For most of history before that, it was around 18.

Ignoring technological differences, the world was a quite different place a few centuries ago, due simply to the difference in life expectancy. Although there were still people at middle, old, and very advanced ages, there were significantly fewer of them. The world was young.

Most authorities agree that the rapid increase in life expectancy of this last century has reached an end, however. Curing cancer would only add about three years, and curing heart disease would add about fourteen – far less together than the twenty-six years added since 1900. And the gains would have to take place at the older ages. The younger ages have already seen most of the improvement we're likely to see.

14.3. Survival Curves and Civilization

For the most part, aging is something new in the world since the arrival of man. Animals in the wild do not die of "old age". They die of disease, accident, predation, or starvation. Aging is an artifact of civilization. The survival curve for animals in the wild is more like that of a group of test tubes in the laboratory or the atoms of a radioactive element. A constant rate of destruction results in a concave, downward-sloping curve. Mankind's survival curve was similar until we began to engage in agriculture and animal husbandry, instituted laws for the common good and protection, built sewage and water systems to control disease, developed antibiotics, and so on.

Today, our mortality rates drop from very high levels at birth to their lowest levels at 11 or 12, and then double every 6-8 years from age 30 on. In extreme old age, those most susceptible to disease have already succumbed. The survivors are a hardy lot, and the mortality rate doubling time slows. The result is the survival curve with which most actuaries are familiar. The gap between this curve and the constant-rate curve represents the difference in mortality attributable to civilization.

Figure 1 illustrates the difference in survival curves for non-aging and aging populations.

Survival Curves

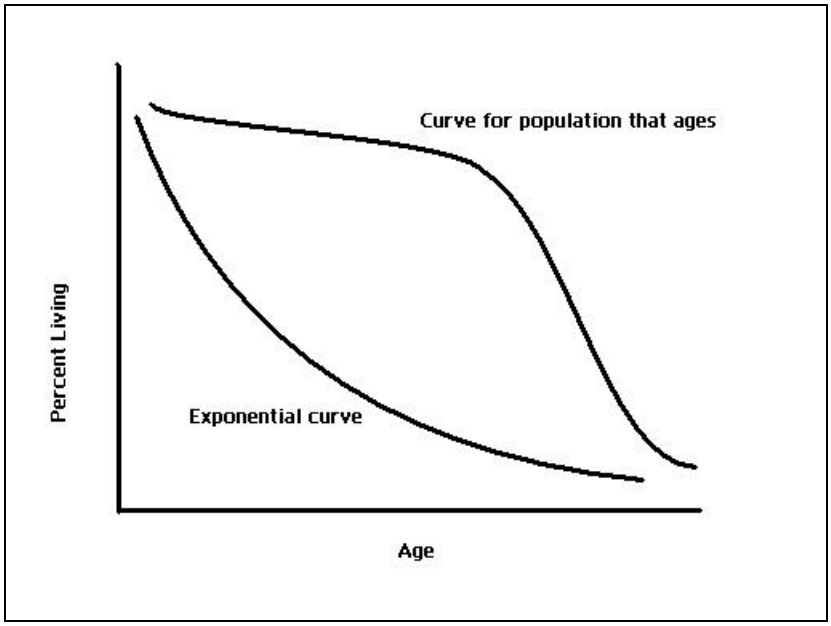


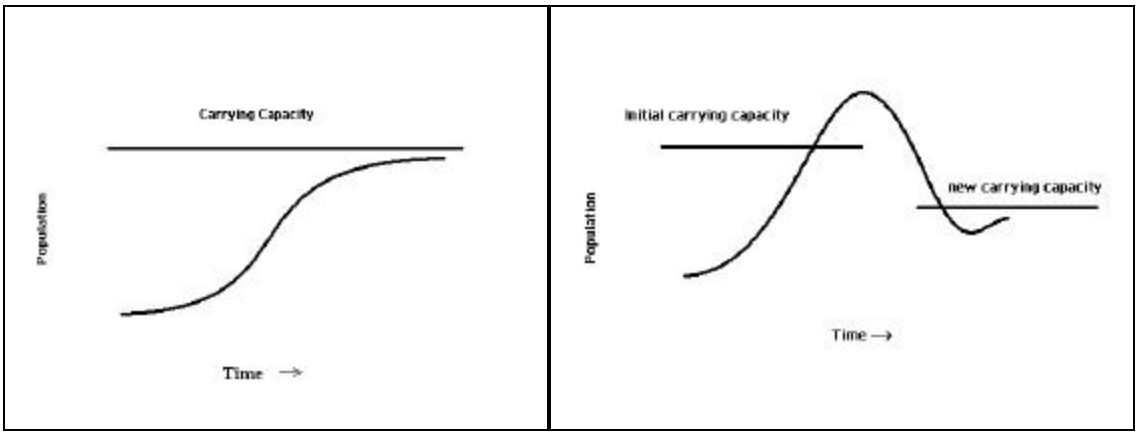
Figure 1.

14.4. Changing the Length of Life

Obviously, if it turns out we are able to postpone the aging process and extend the human life span significantly, it will cause huge upheavals in society. There is no historical precedent for an event of such magnitude. It is a development that would put every institution on the planet through a meat-grinder of change.

Much has been written about the economic and sociological implications of an increasing life expectancy and an increasing population. There are myriad challenges posed by these two events, and they will surely tax our ingenuity to the utmost to deal with them. Rather than examine any one, or group, of issues related to these events, I prefer to make one simple, parting point to the reader, by means of a few graphs.

14.5. Population Growth and Environmental Carrying Capacity



Figures 2a and 2b Illustration of population growth and environmental carrying capacity.

Figure 2 is a set of two graphs illustrating the relationship between population growth and environmental carrying capacity. As the first curve shows, populations of animals typically exhibit a logistical growth pattern, growing rapidly at first, and then slowing as they reach environmental limits on food, water, and so forth. If the population expands too much, a population overshoot occurs, as the second curve shows. This is followed by a population crash, or die-back, to a level sustainable by the environment. The population usually re-stabilizes at a lower level because the environment often is damaged during the crash and is no longer capable of sustaining the original population size.

14.6. Human Population Curve

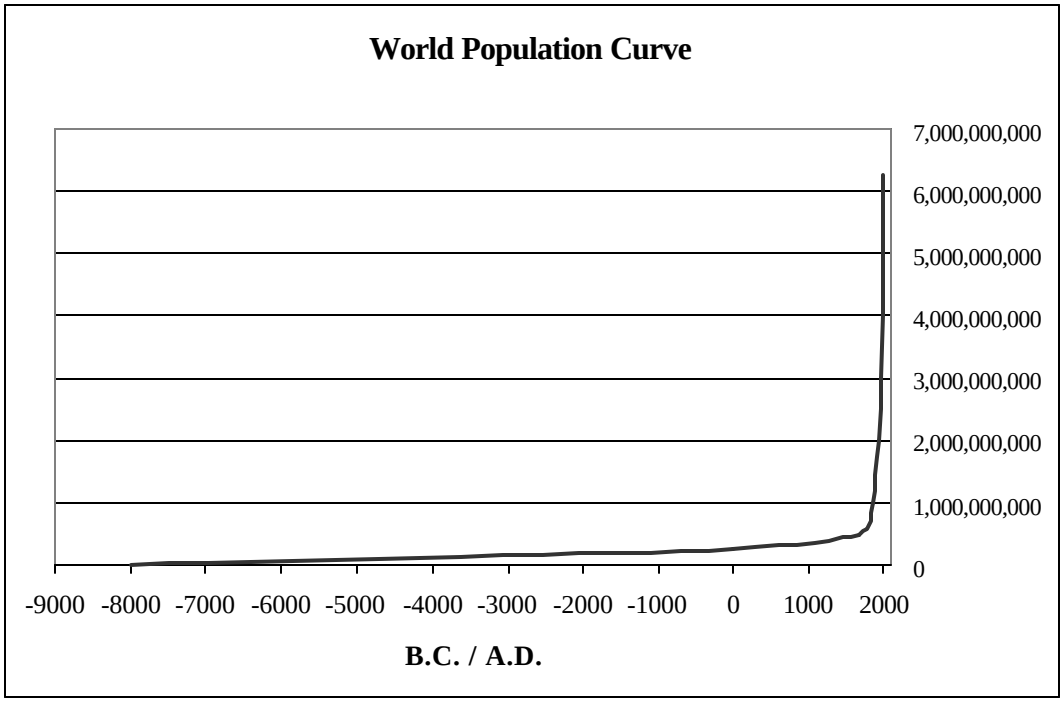


Figure 3. World population for the last 10,000 years.

Figure 3 shows an estimate of the world population for the last 10,000 years. In 8000 B.C. there were about 5 million people worldwide. At the time of Christ, there were roughly 250 million; the approximate size of the U.S. today. Today, there are 6.2 billion, and estimates for 2010 are in the 7.25 billion range.

The juxtaposition of this graph with the previous ones should command one's attention immediately. It is impossible to look at the growth curve for the human population without thinking about the one dealing with carrying capacity. Whatever the ideal population for this planet is, it seems obvious we exceeded it a long time ago. And this is without extending the human life span.

What happens if we throw the gasoline of extended life span on the bonfire of runaway population growth?

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