WORKSHOP

REPORT

The Aging Factor in Health and Disease

The Promise of Basic Research on Aging

INTERNATIONAL

LONGEVITY

CENTER - USA, L TD.

The Aging Factor in Health and Disease

The Promise of Basic Research on Aging

An Interdisciplinary Workshop of the
International Longevity Center-USA, Ltd.

Sponsored by The Brookdale Foundation Group
With Additional support from The Institute for the Study of Aging
and an Educational Grant from Pfizer Inc

New York

February 10-11, 1999

Participants

Steven N. Austad, Ph.D.
University of Idaho

Jacob A. Brody, M.D.
University of Illinois - Chicago

Robert N. Butler, M.D.
International Longevity Center-USA, Ltd.

Judith Campisi, Ph.D.
Lawrence Berkeley National Laboratory

Anthony Cerami, Ph.D.
The Kenneth S. Warren Laboratories, Inc.

Gene Cohen, M.D., Ph.D.
George Washington University

Vincent J. Cristofalo, Ph.D.
Lankenau Medical Research Center
Thomas Jefferson University

David A. Drachman, M.D.
University of Massachusetts Medical Center

Caleb E. Finch, Ph.D.
University of Southern California

Irwin Fridovich, Ph.D.
Duke University Medical Center

Calvin B. Harley, Ph.D.
Geron Corporation

Richard J. Havlik, M.D., M.P.H.
National Institute on Aging

George M. Martin, M.D.
University of Washington

Richard A. Miller, M.D., Ph.D.
University of Michigan

S. Jay Olshansky, Ph.D.
University of Chicago

Olivia M. Pereira-Smith, Ph.D.
Baylor College of Medicine

James R. Smith, Ph.D.
Baylor College of Medicine

Richard L. Sprott, Ph.D.
The Ellison Medical Foundation

Huber R. Warner, Ph.D.
National Institute on Aging

Michael D. West, Ph.D.
Advanced Cell Technology

T. Franklin Williams, M.D.
University of Rochester

John R. Wilmoth, Ph.D.
University of California - Berkeley

Woodring E. Wright, M.D., Ph.D.
University of Texas Southwestern
Medical Center

Observers

Michael Ahlijanian
Pfizer Inc

Felix J. Baker, Ph.D.
Tisch Family Interests

Commander John DuGene
Naval War College

Joe Feczko, M.D.
Pfizer Inc

Howard Fillit, M.D.
Institute for the Study of Aging

Tilman Friedrich, M.D.
Pfizer Inc

Raymond Handlan
Atlantic Philanthropic Service

Nina M. Hill, Ph.D.
Pfizer Inc

Shiro Horiuchi, Ph.D.
Rockefeller University

Mark Horn, M.D.
Pfizer Inc

Laura Johannes
Wall Street Journal

Kazuo Koshiya, Ph.D.
Yamanouchi USA, Inc.

Gregory Stock, Ph.D.
University of California, Los Angeles
School of Medicine

Contents

Preamble

There are many unanswered questions about what causes the adverse effects associated with aging, and even what aging actually is, including the potential for continuing creativity and contributions to society. Answers to these questions will give us powerful insights into the mechanisms of aging and the causes and treatment of age-related diseases. Currently, the losses of function and costs of medical care are concentrated in the last years of life; thus, it is clear that maintaining healthful functioning and reducing the morbidity associated with aging could have a significant effect on overall life satisfaction and cost of medical care in the United States. With these issues in mind, a multidisciplinary workshop was held in New York City on February 10-11, 1999 sponsored by the International Longevity Center-USA to discuss "The Aging Factor in Health and Disease." The goal of this workshop was to identify promising areas of research which could lead to biological interventions to prevent, delay, or reverse the adverse effects associated with aging, in particular the burden of disease.

Assuming that there are both genetic and environmental risk factors for aging, the important general questions become: do these risk factors affect aging and disease independently of each other; do age-related changes make humans more vulnerable to disease; or does disease occur independently, which then hastens aging? Viewed from the perspective of individual tissues, or specific processes, these options may not be mutually exclusive, as what might obtain for one tissue, may not for another. Unfortunately, this means that a complete understanding of aging will require not only an investigation of general processes such as oxidative damage and repair, glycation, changes in gene expression, but also an investigation of these processes on a tissue by tissue basis. It is also important to deduce the actual mechanism(s) by which genetic interventions extend the maximum life span of model organisms, as there are already several examples of interventions of this kind in various model systems (Table 1).

Words used to describe the processes of aging tend to suffer from imprecision. For the purpose of this discussion, we are using Caleb Finch's suggestions (Finch, 1990). Aging will refer to changes that occur during the life span, not all of which need to be adverse. Senescence, on the other hand, refers to "age-related changes in an organism that adversely affect its vitality and functions," and is due to the inevitable passage of biological time. Thus, senescence clearly implies a degenerative process. George Martin suggests the utility of defining a period of the life course called "sageing," as the interval between decline in reproductive fitness and the onset of senescence. During this period a series of adaptive physiological and behavioral changes may occur in response to both intrinsic and extrinsic challenges. Maximum life span will refer to the empirical value observed for the longest surviving individual in a population; it is not a fixed value, and will depend upon environmental conditions. If an organism lives longer in response to some intervention, it may either be senescing more slowly overall, or the intervention may have uniquely prevented some critical degenerative process.

The participants at this workshop discussed what general questions need to be answered for a thorough understanding of both aging and senescence, what approaches appear to be useful for answering these questions, and the need

Table 1

Genes with Significant Effects on Life Spans in Model Systems

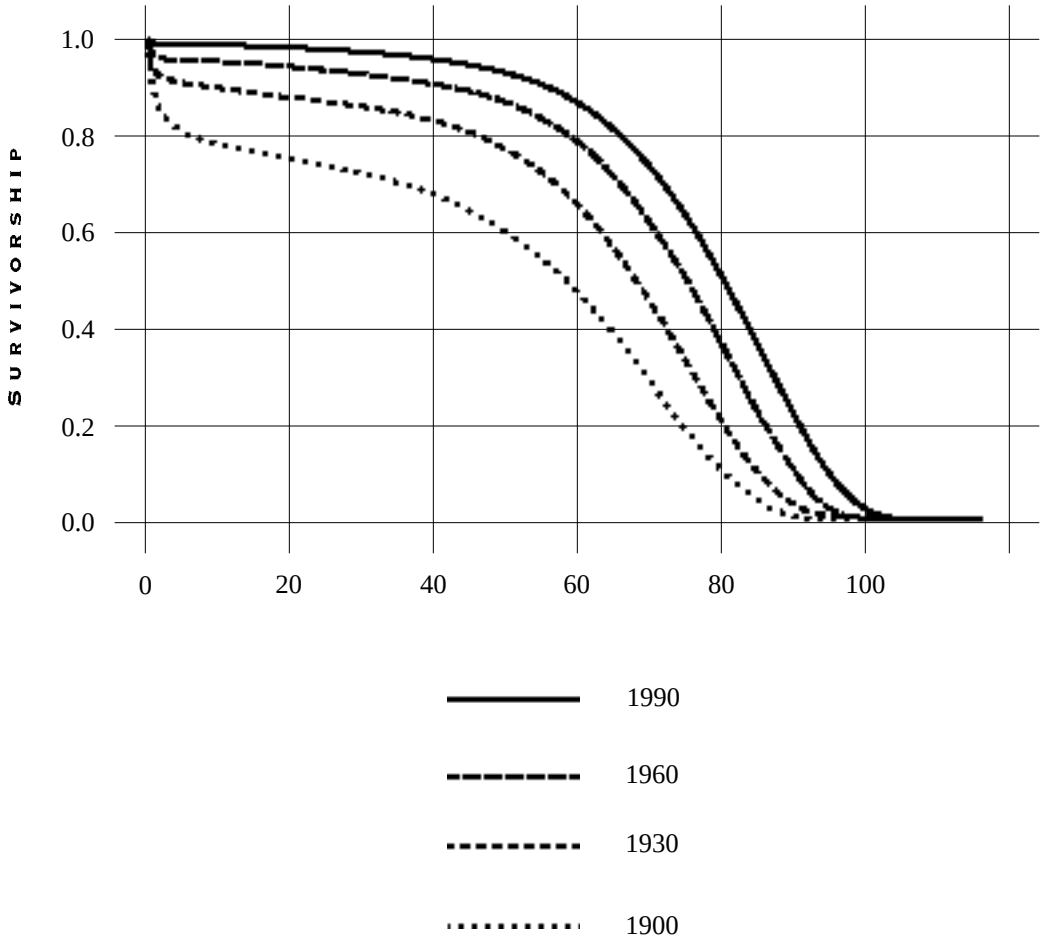
Model System	Gene	Biochemical Function	Comments	Reference
Yeast	V-Ha-*ras*	Viral oncogene	controlled expression of *ras* extends life span 100%	Chen *et al.*, 1990
Fruit fly	*methuselah (mth)*	Homologous to membrane GTP-binding protein	*mth* mutation extends life span by 35%	Lin *et al.*, 1998
Fruit fly	*sod-1*	Cu/Zn-superoxide dismutase	expression of *sod-1* in motorneurons extends life span up to 40%	Parkes *et al.*, 1998
Nematode	*age 1/daf23*	phosphatidyl-inositol-3-kirase	*age 1* mutation extends life span by up to 100%	Morris *et al.*, 1996
Nematode	*daf2*	homologous to human gene for insulin receptor	*daf2* mutation extends life span by at least 100%	Kimura *et al.*, 1997
Mice	thioredoxin	reduce oxidized groups in protein	transgenic overexpression extends life span by 30%	Yodoi *et al.*, 1999
Human cells	*TERT*	catalytic subunit of telomerase	maintains telomere length; extends proliferative potential of cells in culture (indefinitely?)	Bodnar *et al.*, 1998 Jiang *et al.*, 1999

to support the infrastructure, in terms of people and equipment, required for adequate progress in basic gerontological research.[1] There is much new technology being developed which has the potential to help unlock the secrets of aging. However, this will not be an easy task, and it is important to distinguish between hyperbole and genuine scientific progress which underlies efforts to modify the rate of senescence.

The purpose of this document is to highlight and call broad attention to the challenges we face and the urgency of achieving advances in our knowledge, through summarizing some of the promising new opportunities to understand aging. Taking advantage of these opportunities will promote the development of effective interventions to prevent or retard critical adverse aspects of the aging experience, and in so doing improve the quality of life of older Americans.

Figure 1

Period life table estimated survivorship for U.S. population



The Workshop Report

Introduction

In 1825, an English actuary named Benjamin Gompertz observed that death rates in humans rise exponentially after sexual maturity, and he proposed a simple formula to define the relationship between the force of mortality and age within a population. This is often used to characterize the age-related changes in mortality of a genetically heterogeneous population, whether human or animal. The Gompertz curve implies no finite maximum age for a species, strain or population, but does predict decreasing probabilities of survival with increasing age. The crucial question now is: Can the slope of the Gompertz curve be altered by biological interventions, i.e., can basic research on aging provide interventions to improve quality of life by decelerating the rate of senescence.

A different, but simpler way to present survival data is to plot the fraction of individuals remaining in a population as a function of the age of the population, i.e., a survival curve. From such a plot it is possible to: 1) detect early deaths which presumably are not due to the usual senescence - related changes; 2) determine the median age of death of the individuals within the population; and 3) estimate the maximum life span of the species, i.e., an age at which the chance of survival of any individual is negligibly low. The past century has been characterized by large decreases in early deaths caused by infectious diseases, and a dramatic increase in the average life expectancy of humans due to improvements in sanitation, health care, housing, nutrition, the development of vaccines, and the discovery of antibiotics (see Figure 1). Substantial future progress will depend upon obtaining a much better understanding of what aging is, particularly the adverse components, and how to intervene to either prevent, reverse or retard these adverse age-related changes.

As a result of the striking increase in the life expectancy of Americans in the 20th century, the United States of America is now anticipating a doubling of Americans aged 65 or older, from 35 million in 2000 to at least 69 million in 2020. This doubling in the number of individuals aged 65 or more could bring with it large increases in disability, loss of function, and the cost of health care for this segment of the population. Thus, it is imperative that the biomedical community make substantial advances in reducing the major causes of disability in the elderly population. This can only occur with appropriate levels of investment in aging research. Edward Schneider estimates that the United States currently spends only 0.3% as much on aging research as it does on health care services for older people (Schneider, 1999). Money spent on research to better understand the mechanisms of aging and the causes of age-related disease may lead to interventions to both postpone the need for aging-related health care, and partially pay for itself in reduced health care costs. Whether these practical benefits will start to accrue in years or in decades will depend on the vigor of the investment.

Aging vs. Age-related Pathology: The Role of Age-Related Changes

A continuing debate has been whether a pattern of aging free of disease can be dissected away from the overlapping development of age-related dis-

eases such as cancer, cardiovascular disease, osteoporosis, osteoarthritis, diabetes, and a large variety of neurodegenerative diseases, including Alzheimer disease. The most substantial attempt to do this has been the 40 year old Baltimore Longitudinal Study on Aging (BLSA), which has attempted to describe age-related changes by making successive measurements over a period of time. Only apparently healthy individuals are accepted into the study. Whereas many facts have emerged from this study, the foremost is that the effects of aging are extremely variable from person to person, so it is almost impossible to evaluate the "aging status" of an individual based on one, or even a few, biochemical, physiological or physical measurements. The implication of this is that it has not yet been possible to develop a panel of human biomarkers which can reliably estimate the biological age of an individual, as opposed to the chronological age. A research initiative to develop a panel of biomarkers in rodents (mice and rats) has produced a few promising leads, but has not yet produced a panel of validated, reliable tests.

Another observation from the BLSA is that most measurements show gradual changes with age, rather than precipitous changes. It is thus assumed that precipitous changes are more likely to be associated with the development of specific age-related pathology. Jacob Brody and Edward Schneider have previously identified the occurrence of at least two distinct types of associations; aging-dependent and age-dependent (Brody and Schneider, 1986). In particular, there are certain genetically determined changes leading to deficits which are age-dependent, i.e., they express themselves at predictable ages, which are apparently not normal aging, e.g., Huntington disease. However, age-related changes that are associated with normal aging also contribute to the development of pathology. Thus, the immediate challenge is to distinguish among:

1) pathologically neutral age-related changes, e.g., graying of hair;
2) changes which may contribute to the development of one or more age-related pathologies, e.g., accumulation of oxidative damage; and
3) changes which may cause or indicate overt pathology, e.g., the development of plaques and tangles in the brain as risk factors for Alzheimer disease. Distinguishing among these possibilities is necessary to guide and prioritize the development of anti-senescence interventions.

The longer term challenge is to understand the molecular bases for the *rate* at which species senesce, and individuals within a species senesce, and ultimately to develop strategies to decrease that rate. This has already been accomplished to some extent in simple animal models, using death as the endpoint. In some cases life span has been extended several fold, although it is not clear whether this can be construed to mean that the rate of senescence in these model systems has been modified. Success in extending the maximum life span of mammalian models has been much more modest, and has been achieved mainly through caloric restriction. So far with humans, longevity has been increased through the prevention and treatment of life-threatening disease.

By now it is clear that some age-related changes are risk factors for disease, and that these changes can be both intrinsic and extrinsic, and either random or programmed (genetic). Some of what we already know about these risk factors is summarized in the next two sections.

Intrinsic Stochastic Factors in Aging

It is now well established that aging is very "plastic" in the sense that individual humans,

and other animals, display individually different patterns of change during aging. This implies that environmental factors, both negative and positive, have an impact. Research during the past 25 years has identified a number of negative factors such as oxidative damage to proteins, DNA and lipids; glycation; changes in protein conformation; and induction of mutations. Whereas cells have robust repair systems to reverse many of these processes, repair is seldom complete or instantaneous.

Oxidative damage is an intrinsic process in organisms which require oxygen for life, as damaging oxygen radicals are continuously produced during cellular respiration. Although it is reasonable to assume that some persisting oxidative damage is acceptable, particularly if it occurs in non-critical molecules or molecules present in great excess, unrepaired damage to molecules involved in critical rate-limiting processes are likely to have negative effects during aging. Thus, it is not surprising that there are several examples of extension of life span by over-expression of genes coding for anti-oxidant enzymes (Table 1).

Glycation is another intrinsic process which is unavoidable in most animals. Glycation involves a non-enzymatic reaction between the carbonyl group of a reducing sugar, usually glucose, and the primary amino groups of proteins. This initial reaction to form a Schiff's base is usually followed by a complex series of reactions, most of which are poorly characterized, often leading ultimately to protein cross-linking. This overall process not only alters the structure and function of the proteins themselves, but also leads to more global effects such as blood vessel wall and connective tissue stiffening. Several compounds are currently in development to either prevent or reverse this kind of damage.

Other structural changes occur during aging with possible negative implications. For example, the conformation of individual proteins may change, leading to loss of function, and sometimes aggregation. Changes in the aggregation state of specific proteins have long been associated with Alzheimer disease and related neurodegenerative disorders (Hardy and Gwinn-Hardy, 1998); whether there is a causal relationship is still a matter for debate.

Finally, the importance of apoptosis during aging is now recognized (Warner et al., 1997). Apoptosis is a genetically regulated, inducible cell death program. It is known that apoptosis is induced by oxidative stress and DNA damage, but the exact induction pathway is not known. When apoptosis occurs in post-mitotic tissue such as the central nervous system, the result may be catastrophic, as in the case of neurodegenerative diseases such as Alzheimer disease, Parkinson disease, Huntington disease, etc. On the other hand, apoptosis appears to play an important role in reducing cancer.

Genetic Factors in Aging

Recent technological advances can now provide unprecedented opportunities to understand the genetic basis of aging, and with that the development of interventions to improve the quality of life in older persons. These advances include the ability to: 1) isolate and obtain the sequence of individual genes; 2) generate genetically altered mice, e.g., transgenic mice, to characterize the functional role of such genes; 3) develop sensitive and rapid techniques to measure the expression of thousands of genes in a single assay; 4) detect small differences (polymorphisms) in the DNA sequence of any gene among individuals in a population; and 5)

determine the entire sequence of the genome of humans, as well as that of useful animal models. It is anticipated that the sequence of the human genome may be accomplished by 2003, or perhaps sooner. The complete sequences of popular model organisms such as *Caenorhabditis elegans* (a nematode), *Saccharomyces cerevisiae* (a yeast), and *Escherichia coli* (a bacterium) have already been determined (Goffeau et al., 1996; Blattner et al., 1997; the *C. elegans* sequencing consortium, 1998). These analytical techniques, and the knowledge which can be obtained with their appropriate use, will allow the biomedical community not only to evaluate the effectiveness of a wide variety of biological interventions, but also to determine the genetic basis for the wide diversity in the individual patterns of aging.

The observation that a strain of long-lived fruit flies can be derived from an outbred population of flies (Rose, 1984), indicates that genetic factors do play a role in aging. The case has been made best in short-lived model organisms, such as yeast, nematodes, fruit flies and mice. A list of genes (Table 1) implicated in regulating longevity and aging includes genes for proteins with anti-oxidant activities, e.g., superoxide dismutase, catalase, and thioredoxin; and signal transduction proteins, e.g., insulin receptor, phosphatidyl inositol-3-kinase, ras and GTP-binding protein (the *methuselah* gene product). All of these genes have human homologs. A case has also been made for a genetic role in human longevity (Herskind et al., 1996); based on a study of Danish twin pairs, these authors estimated that the longevity in humans is about 25% due to genetic factors.

It is not yet known whether the ability to prevent replicative senescence and extend life span at the cellular level, by inducing the expression of telomerase, will have a similar effect at the organismal level. Telomerase deficiency in mice does lead to some pathology due to reduced proliferative potential (Rudolph et al., 1999), as the telomeres gradually shorten in successive generations. In particular, these mice suffer from slow wound healing, ulcerative skin lesions, reduced ability to respond to immune system stress, intestinal pathology, and reduced longevity. Although some of these changes are characteristic of human aging, the full spectrum of age-related diseases is not seen. These mice are cancer-prone, a trait apparently linked to genetic instability caused by critical telomere loss. It remains to be determined how a lack of telomerase will affect progression of rapidly growing tumors.

The Need for Animal Models

Whereas the ultimate goal is to understand human aging, many experiments are too invasive to be performed on humans. Furthermore, the long life span, genetic heterogeneity, and diversity of environmental exposure of humans make them unsuitable for many kinds of experimentation. Thus, animal models have been used extensively to study normal aging (Sprott and Austad, 1996). Mice have been a particular favorite because of their short life span, well-characterized genetics, easy husbandry and low cost. Also, they can be easily raised in a pathogen-free facility. For similar reasons, except for the relative paucity of genetic information, the rat is also a favored model, especially because of its larger size. Neither the mouse nor the rat genome has yet been sequenced, but emphasis is now being placed on obtaining the mouse sequence (Battey et al., 1999). It is clear that much of the pathology

occurring in mice and rats is similar to that found in humans, although Alzheimer disease and stroke are not found in old mice and rats. However, human pathology can often be reproduced in genetically altered mice, and the popularity of mice has increased dramatically because of this ability to create transgenically altered mice which mimic human diseases.

Almost all studies done on mice and rats so far have been done using genetically homogenous strains. This made sense in early gerontological studies focused mainly on characterizing species-specific aging phenomena. However, the recent emphasis on identification of genetic factors which influence life span and the rate of aging can benefit from the use of genetically heterogeneous models (McClearn, 1997). No commercial colony of such a model currently is available, but preliminary experiments with several institution-based colonies suggest that they may indeed be useful for identification of genetic loci with significant effects on life span (Harrison and Broderick, 1997; Miller et al., 1998). One unique value of such a colony is that it more closely reflects the genetic diversity of human populations, and thus provides an opportunity to ask questions about genetic associations with age-related phenotypes.

However, the above mammalian models have several limitations. Although these rodents do have fairly short life spans (two to four years, depending on strain), these animals are too long-lived to permit routine screening for long-lived mutants, as has been done with nematodes and fruit flies.

Also, these rodents rarely develop atherosclerosis or neurodegenerative disease, and mice have very high incidence of cancer. Because of this, there is a real need to develop a non-human primate model to use as a better surrogate for human aging. Although rhesus monkeys are currently being used in experiments to determine whether the extension of life span and retardation of age-related disease by caloric restriction might work in humans (Couzin, 1998), these monkeys are fairly long-lived (up to 40 years). Thus, small, relatively short-lived prosimians, e.g., lemurs, or new world monkeys, e.g., marmosets, with life spans in the range of 12 to 20 years have been suggested as a more suitable model of human aging, but no colonies are currently available for such use.

Another underutilized potential animal model for studying aging is the avian model (Holmes and Austad, 1995). Birds are highly aerobic and have high circulating glucose levels, but despite these characteristics, they frequently live longer than similar sized mammals. It would be useful to know how various bird species overcome the apparent disadvantages of high glucose concentration and high rate of oxygen metabolism. Thus, despite the relative paucity of genetic information about bird species, there is a need for standardized, accessible bird colonies for those wishing to tackle these gerontologically important questions.

Finally, it can be asked what human populations provide opportunities for studying human aging which are not yet being fully utilized. There appear to be several. These include groups with small founder populations, sib pairs and twins, large family groups for which it is possible to obtain substantial clinical data, and individuals who display successful aging phenotypes. These latter include very old individuals, e.g., centenarians, who are relatively free of disease and dis-

ability, and older individuals who are particularly fit and healthy, e.g., master athletes. DNA samples and relevant clinical data could prove extremely useful for establishing association between genes and aging-related phenotypes in these human populations.

New Tools and Resources for Aging Research

The application of molecular biological techniques to studies in experimental gerontology has led to remarkable advances in understanding the genetic basis of aging in invertebrate animal model systems. These have included the identification of several genes which alter the rate of aging in nematodes (Morris et al., 1996; Kimura et al., 1997), and fruit flies (Lin et al., 1998; Parkes et al., 1998). The production and characterization of genetically altered mice (transgenic and gene knockout mice) are also providing useful animal models for the study of age-related pathology (Williams et al., 1998; Morgan et al., 1999), and age-related diseases such as Alzheimer disease (Hsiao et al., 1996). These kinds of approaches are generating important information about the genetic basis of aging in these particular animal models, and it is hoped, relevant insights about human aging. Examples of these genes are shown in Table 1.

Other new technologies, which will provide even greater insights into both species-specific aging and the genetic basis of aging, are now becoming available. These include: 1) the polymorphisms, including single nucleotide polymorphisms, in any gene of known sequence, and the possibility of demonstrating an association between any particular polymorphism and an aging-related phenotype; 2) use of gene expression microarrays to characterize gene expression patterns in any tissue in any species of interest, as a function of age, and under a variety of conditions, especially in response to possible anti-senescence interventions; and 3) advances in technology for high-throughput DNA sequencing (Lander, 1999; Chakravarti, 1999). Unfortunately, these techniques require expensive equipment and related materials which are not yet available in most laboratories. Thus, there is a need to provide the equipment and infrastructure needed to use these technologies, and/or provide support for these technologies using microarray chips and filters produced commercially. This includes the need for an efficient data management system to permit ready access by interested laboratories to permit data sharing, and thus avoid expensive duplication of effort.

The ability to characterize patterns of gene expression in animal model systems is only the first step in understanding the significance of age-related changes in gene expression in humans. Information about such patterns might provide useful biomarkers of aging. However, in order to obtain baseline data in humans, a bank of human surgical and autopsy material will be required. Such material must be very carefully collected and maintained to be of any use for study of gene expression patterns, because RNA is very unstable in such tissues after death. Such a bank should contain samples of all major human tissues, collected over a wide range of ages, from healthy individuals with well-characterized clinical histories. Samples should also be annotated with a thorough pathological analysis obtained at the time the autopsy samples are obtained. Similar tissue banks will be needed for the model systems chosen for animal studies.

Avenues and Specific Goals of Basic Research on Aging

The conviction that basic research on aging is underfunded comes from the recognition that there are many unanswered questions about the basic mechanisms of aging, and that the answers to these questions may give us powerful insights about aging and the treatment of age-related diseases. The following goals are examples of research questions which need to be answered in our desire to understand the mechanistic basis of age-related changes.

- Identification of the Genetic Differences which are Causally Associated with Life Span Differences Between Species.

The genes responsible for the dramatic differences in longevity among animal species are currently unknown, even between species that are closely related. The identification of these genes and demonstration of their function *in vivo*, would be a significant accomplishment for understanding the genetic basis of senescence in general.

- Generation of Animal Models with Significantly Postponed Senescence, and Identification of the Genes Responsible for the Life Span Extension.

The generation of mice with a significantly extended life span, and identification of the genes responsible, will continue to be an important goal, because it is likely that such information will provide insights into the genetic basis of senescence in humans.

- Identification of Linkage Between DNA Polymorphisms and Longevity and/or Age-related Pathology.

Longevity differs dramatically among individuals of the same species. These differences are assumed to be due to differences among individuals due both to their environment and their genomes, but are also related to chance events during development and aging. Any association study linking genomic differences to either longevity or age-related pathology would provide insight about the genetic basis of senescence either in humans or the specific animal model being studied.

- The Role of Oxidative Damage in Senescence.

It is widely assumed that some forms of age-related pathology result, at least in part, from oxidative damage. The unrealized challenge is to tack real numbers onto this vague generalization; i.e., what proportion of dysfunction, in which cells and tissues, is really due to oxidative damage? Are there critical proteins which are oxidized, thereby compromising overall cellular and tissue function? Another challenge is to develop interventions that either reduce the rate of production of oxygen free radicals or increase the activity of anti-oxidant defense systems. Interventions that consistently slow the rate of senescence in model systems would suggest promising clinical trial opportunities in human populations, especially in delaying degenerative changes in post-mitotic tissues.

- Caloric Restriction.

Although it has been known for more than 60 years that caloric restriction extends the maximum life span of most animal species in

which it has been adequately tested, the mechanism by which it does so is not known for any species. Research to elucidate the causal mechanism(s) could suggest surrogate interventions to retard senescence without actually restricting calories.

- BIOMARKERS OF AGING.

The lack of a panel of biomarkers of aging means that there are few, if any, indicators of the rate of aging in any particular individual. Such a panel is needed to test promising interventions, first in animal models, and eventually in the human population, because measuring survival is a very inefficient and crude alternative. The particular challenge is to demonstrate an empirical link between putative biomarkers of aging, which can be measured continuously over the life span of an individual, and the timing of *adverse* events, including death or the onset of disease or disability. Reversing these events is the ultimate goal of interventions.

- WERNER SYNDROME: A HUMAN MODEL OF RAPID SENESCENCE.

Patients with Werner syndrome (WS), develop many of the adverse phenotypes of aging, but at an accelerated rate. *WRN*, the gene defective in WS, has now been cloned and shown to code for a protein with both helicase and exonuclease activity. However, there are at least five other helicase genes with related functions in the human genome. The role of the *WRN* gene product and related proteins in retarding senescence in humans is not yet known, but they could be involved in any or all of the following: DNA replication, DNA repair, transcription, recombination, or chromosome segregation. Fibroblasts from WS patients, which have reduced proliferative capacity, also lose telomeric DNA significantly faster than normal fibroblasts, implicating the *WRN* protein in telomere dynamics as well. Elucidation of the actual role of the *WRN* protein *in vivo* could provide significant insight into the causes of several age-related diseases such as cancer, cardiovascular disease and cataract. For example, a common DNA polymorphism in *WRN* is a risk factor for myocardial infarction (Ye et al., 1997).

- CELLULAR SENESCENCE *IN VIVO*.

Since the finding by Leonard Hayflick that human cells do not divide indefinitely when grown in culture, cultured human cells, principally fibroblasts, have been a much-used, but controversial model for human aging. It is now known that when human cells lose their ability to divide, they also lose functional capacity, in the sense of acquiring new and deleterious activities, although they do not die. This has been termed the cellular senescent phenotype. This loss of proliferative and functional capacity can be induced by telomere shortening, certain types of DNA damage, and some oncogenic stimuli. Preliminary data showing that senescent cells do exist *in vivo* (Dimri et al., 1995), suggest that accumulation of such cells could have negative implications for the integrity of the extracellular matrix in the vicinity of such cells (Campisi, 1997).

Replicative senescence in human fibroblasts can be prevented by transgenic expression of the catalytic subunit of telomerase (Bodnar et al., 1998). Such cells become capable of continued proliferation well beyond their usual limit. This suggests the potential to reverse any *in vivo* phe-

notype which might be due to loss of proliferative capacity, e.g., wound healing, immune senescence, provided telomerase activity can be selectively restored in the cells of interest.

- CELL REPLACEMENT AND STEM CELLS.

Cells in which the telomerase gene has been reactivated are possible candidates for use in replacing cells lost through apoptosis due to excessive damage, wear, or trauma. Fortunately, it is now apparent that such cells are not necessarily carcinogenic even though they express telomerase (Jiang et al., 1999). An alternative approach is the use of stem cells which retain pluripotent capability. Such cells have recently been isolated, and have tremendous potential to treat a variety of age-related degenerative diseases (Gearhart, 1998). However, there remain many technical and conceptual hurdles to control the differentiation of such cells once they have been transplanted. If, and when, these hurdles can be overcome through research, applications of this technology to reverse the adverse effects of aging appear to have significant potential.

- HORMONE REPLACEMENT.

While it is seductive to believe that restoration of hormone levels in older individuals to the levels found in young individuals will reverse aging, this may or may not be true. Thus, while research to determine the effects of manipulating hormone levels has enormous potential, e.g., estrogen replacement therapy, the general question is complex because hormones have both positive and negative effects, and it may be difficult to adequately mimic the natural daily variations of each hormone. Much research is needed in this area to determine which hormone replacement therapies are both effective and safe (Lamberts et al., 1997). For example, preliminary studies with growth hormone (GH) indicated that muscle mass and a general feeling of well-being are increased by GH (Rudman et al., 1990), but safety remains an important concern as serious side effects may occur.

The above examples give credence to the idea that research on aging has tremendous potential to raise our level of understanding enough to lead to successful interventions in both aging, and specific age-related diseases. Any delay or reduction in age-related disability and disease will increase the number of years of healthy life and is a worthy goal.

To facilitate such progress in understanding aging there is a critical need not only for increased funding for aging research, but also to provide funding for research infrastructure in the form of equipment and trained personnel.

Footnote

1 The research goals articulated in this document are a product of both the discussions in the ILC workshop held in New York City, February 10-11, and a workshop recently sponsored by the UCLA Program on Medicine, Technology and Society, and convened by Dr. Gregory Stock.

This latter meeting held in Los Angeles, which extended the discussions begun in New York involved several attendees at the ILC conference, as well as Drs. Michael Rose, Cynthia Kenyon, Jan Vijg and James Nelson.

Commentary

BY ROBERT N. BUTLER, M.D.
AND T. FRANKLIN WILLIAMS, M.D.

Together we had the responsibility (and the pleasure) of helping establish and direct the National Institute on Aging over nearly two decades, during its formative period between 1975 and 1991. We witnessed the remarkable advances in the understanding of the basic biology of aging and age-linked diseases as well as of the social and behavioral aspects of aging. Although funding for the Institute has grown considerably from an original $12 million to nearly $500 million annually, there remain many challenging research opportunities, as well as needs of the Institute's various programs. We personally favor increasing support for the NIA in general, but in this document our focus is upon the opportunities posed by the intellectual vigor of modern biology. We believe insufficient funds are available to study the molecular and cellular processes that define the biology of aging, for example, the fact that the "force of mortality" rises with the passage of time, as delineated by Benjamin Gompertz 175 years ago.

For this reason, we sought financial support for a workshop to explore the impact of aging factors in health and disease, to evaluate the state of knowledge and to consider issues of science policy and funding. *The Aging Factor in Health and Disease: The Promise of Basic Research on Aging* is the result of a two-day workshop supported by the The Brookdale Foundation Group, the Institute for the Study of Aging, and Pfizer, Inc. We are exceptionally indebted to Dr. Huber Warner of the National Institute on Aging for his comprehensive and insightful narrative. We are grateful to the outstanding contributors to American gerontology, in fields ranging from cell and molecular biology to chemistry to demography and epidemiology, who despite other pressing demands made time to participate.

We believe this state-of-the-art scholarly report should become a campaign document to encourage further support of basic biological studies of aging by government, foundations, individual philanthropists and the for-profit sector. These potential supporters should appreciate the growing importance of the demographic revolution as we come to the close of the century and the millennium. The United States now enjoys the highest life expectancy in its history - 76.5 years, while at the same time benefiting from dramatic drops in disability rates. There have been exciting developments such as the cultivation of pluripotent cells, identification of molecular time clocks in aging (telomeres), nuclear transfer or cloning, and the introduction of a variety of significant theories, all of which should be carefully evaluated. There is the promise of important steps in the prevention and treatment of age-related disorders. The prospects for "regenerative medicine" and genomics lie just before us. It is time to dramatically increase the National Institutes of Health's investment in aging research, now only about $100 million out of the $15 billion budget of the NIH.

Arguably, if it were possible to compress morbidity - both in length and degree - biomedical research in general, and aging research in particular might be credited with the resulting reduced health costs. Added research funding might even pay for itself in health cost savings.

A recent examination of disability rates shows a significant 2.1% decline since 1982. Consequently, there are some 1.2 million fewer disabled persons than were anticipated. This reduces Medicare expenses and, of course, more importantly, advances quality of life.

Additional funding for investigator-initiated research, programs and centers should be supplemented with new investments in animal models, laboratory resources, research training and banking of human tissues. The search for an appropriate panel of biomarkers is essential. At present there is no single set of biomarkers to evaluate the many interventions sold in the marketplace under the misleading term "anti-aging medicine." Contemporary enthusiasm for healthful, satisfying longer life must be matched by new funding and critical science - it will not be found in the health food store.

Literature Cited

Battey J, Jordan E, Cox D, Dove W. 1999. An action plan for mouse genomics. Nature Genetics 21: 73-75.

Blattner FR, Plunkett G, Bloch CA, et al. 1997. The complete sequence of *Escherichia coli* K-12. Science 277: 1453-1474.

Bodnar AG, Ouellette M, Frolkis M, et al. 1998. Extension of life-span by introduction of telomerase into normal human cells. Science 279: 349-352.

Brody JA, Schneider EL. 1986. Diseases and disorders of aging: An hypothesis. J. Chron. Dis. 39: 871-876.

Campisi J. 1997. Aging and cancer: The double-edge sword of replicative senescence. J. Am. Ger. Soc. 45: 482-488.

Chakravarti A. 1999. Population genetics - making sense out of sequence. Nature Genetics 21: 56-60.

Chen JB, Sun J, Jazwinski SM. 1990. Prolongation of the yeast life span by V-Ha-*ras* oncogene. Molec. Microbiol. 4: 2081-2086.

Couzin K. 1998. Low-calorie diets may slow monkey's aging. Science 282: 1018.

Dimri GP, Lee X, Basile G, et al. 1995. A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. Proc. Natl. Acad. Sci. USA 92: 9363-9367.

Finch CE. 1990. Longevity, senescence and the genome. The University of Chicago Press. Chicago, IL.

Goffeau A, Barrell BG, Bussey H, et al. 1996. Life with 6000 genes. Science 274: 563-567.

Gearhart J. 1998. New potential for human embryonic stem cells. Science 282: 1061-1062.

Hardy J, Gwinn-Hardy K. 1998. Genetic classification of primary neurodegenerative disease. Science 282: 1075-1079.

Harrison DE, Broderick TH. 1997. Selection for maximum longevity in mice. Exp. Gerontol. 32: 65-78.

Hershkind AM, McGue M, Holm NV, et al. 1996. The heritability of human longevity: A population-based study of 2872 Danish twin pairs born 1870-1900. Hum. Genet. 97: 319-323.

Holmes D, Austad S. 1995. Birds as animal models for comparative biology of aging: A prospectus. J. Gerontology 50A: B59-B66.

Hsiao K, Chapman P, Nilsen S, et al. 1996. Correlative memory deficits, AB elevation, and amyloid plagues in transgenic mice. Science 274: 99-102.

Jiang XR, Jimenez G, Chang E, et al. 1999. Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nature Genetics 21: 111-114.

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. 1997. *daf-2* an insulin receptor like gene that regulates longevity and diapause in *Caenorhabditis elegans.* Science 277: 942-945.

Lamberts SWJ, van den Beld AW, van der Lely A-J. 1997. The endocrinology of aging. Science 278: 419-424.

Lander ES. 1999. Array of hope. Nature Genetics 21: 3-4.

Lin Y-J, Seroude L, Benzer S. 1998. Extended life-span and stress resistance in the *Drosophila* mutant *methuselah.* Science 282: 943-946.

McClearn GE. 1997 Heterogeneous reference populations in animal research on aging. ILAR J. 38: 119-123.

Miller RA, Chrisp C, Jackson AU, Burke D. 1998. Marker loci associated with lifespan in genetically heterogeneous mice. J. Gerontology 53A: M257-M263.

Morgan WM, Richardson A, Sharp ZD, Walter CA. 1999. Application of exogenously regulatable promoter systems to transgenic models for the study of aging. J. Gerontology 54A: B30-B40.

Morris J, Tissenbaum HA, Ruvkun G. 1996. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans.* Nature 382: 536-539.

Parkes TL, Elia AJ, Dickinson D, et al. 1998. Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons. Nature Genetics 19: 171-174.

Rose MR. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster.* Evolution 38: 1004-1010.

Rudman D, Feller AG, Nagraj HS, et al. 1990. Effects of human growth hormone in men over 60 years old. N. Engl. J. Med. 323: 1-6.

Rudolph KL, Chang L, Lee H-W, et al. 1999. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96: 701-712.

Schneider EL. 1999. Aging in the third millenium. Science 283: 796-797.

Sprott RL, Austad SN. 1996. Handbook of the Biology of Aging, 4th Edition. Academic Press, Inc. pp. 3-23.

The *C. elegans* sequencing consortium. 1998. Genome sequence of the nematode *C. elegans:* A platform for investigating biology. Science 282: 2012-2018.

Vlassara H, Fuh H, Makita Z, et al. 1992. Exogenous advanced glycosylation products induce complex vascular dysfunction in normal animals, a model for diabetic and

aging complications.
Proc. Natl. Acad. Sci. USA 89: 12043-12047.

Warner HR, Hodes RJ, Pocinki K. 1997.
What does cell death have to do with aging?
J. Am. Ger. Soc. 45: 1140-1146.

Williams MD, Van Remmen H, Conrad CC, et al. 1998. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice.
J. Biol. Chem. 273: 28510-28515.

Ye L, Miki T, Nakura J, et al. 1997.
Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population.
Am. J. Med. Genet. 68: 494-499.

Yodoi J, Hirota K, Oono T, et al. 1999.
Redox regulation of signal transduction pathways by thioredoxin superfamily.
1999 World Congress on Oxidants and Antioxidants in Biology, p. 45 (Abstr.).

Glossary

AGING - changes that occur during the life span, not all of which need to be adverse.

ALZHEIMER DISEASE - an aging-dependent disease characterized by loss of memory. Risk factors include both genetic and environmental factors. Age of onset varies from the late 40s for patients with early-onset genetic risk factors, to 65 and older for most other patients.

AMINO GROUP - the reactive group found in amines, composed of a nitrogen atom and two hydrogen atoms (i.e., NH2); reacts with carbonyl groups to form a Schiff's base during glycation.

APOPTOSIS - a genetically regulated program leading to cell suicide.

BIOMARKER (OF AGING) - an age-related change which reflects the physiological age of an individual, in contrast to its chronological age.

CARBONYL GROUP - the reactive portion of sugar molecules such as glucose; composed of a carbon atom and an oxygen atom; combines with an amino group to form a Schiff's base during glycation.

CATALASE - an enzyme which destroys hydrogen peroxide, converting it to water and oxygen.

DNA REPLICATION - the process of copying DNA to make two identical copies before cell division occurs.

DNA POLYMORPHISM - a difference in DNA structure found in a small, but significant segment of a given population (in contrast to a mutation which is rare).

FIBROBLAST - one of the major cell types found in human skin; fibroblasts have been developed as a model system for studying cellular aging.

GLYCATION - the non-enzymatic reaction between a reducing sugar and the amino group of proteins; often leads to protein crosslinking.

HIGHTHROUGHPUT (TECHNOLOGY) - technology permitting the assay of thousands of samples simultaneously without individual handling.

HUNTINGTONDISEASE - an age-dependent genetic disease due primarily to the loss of neurons in the striatum; age of onset is about 40 years.

LIFE EXPECTANCY - the average number of remaining years an individual can expect to live at any given age.

LIPID - a class of molecules which are not water-soluble, and form the major component of biological membranes.

LONGEVITY - the length of life of an individual, or the average length of life of a population of individuals.

MUTATION - base substitution in DNA which occurs infrequently within a population of normal individuals (in contrast to a polymorphism which may occur more frequently in a population).

NEMATODE - a small worm, usually soil-dwelling, which has been developed for biomedical research because of its well characterized developmental program; it is a useful model system for studying aging because of its short life span.

NON-ENZYMATIC - not catalysed by enzymes.

ONCOGENIC - causing cancer.

OXIDATIVE DAMAGE - changes in structure of biological molecules due to their interaction with oxygen radicals.

OXYGENRADICAL - a very reactive form of oxygen, produced continuously in mitochondria during normal metabolism.

PHENOTYPE - the visible properties of an organism resulting from the interaction between its genes and the environment.

RECEPTOR - a multimolecular protein complex, usually found on the surface of cells, which binds specific extracellular signalling molecules, thereby transducing a signal to the inside of the cell.

RECOMBINATION - the process whereby two distinct pieces of DNA exchange genetic material.

SCHIFF'S BASE - the product of an interaction between an amino group and a carbonyl group.

SENESCENCE - age-related changes in an organism that adversely affect its vitality and functions.

SUPEROXIDE DISMUTASE (SOD) - an anti-oxidant enzyme which converts the superoxide anion to hydrogen peroxide.

TELOMERASE - an enzyme that synthesizes telomeric DNA.

TELOMERE - the non-coding DNA at the ends of chromosomes consisting of long stretches of short repetitive DNA sequences.

THIOREDOXIN - a protein capable of reversing oxidative damage to other proteins.

TRANSCRIPTION - the process of transferring genetic information from DNA to RNA by copying the DNA sequence.

TRANSGENE - a gene from one organism inserted into the genome of another organism (usually refers to mice).

TRANSLATION - the process of joining animo acids together in specific sequence to form proteins.

WERNER SYNDROME - a genetic disease characterized by premature development of adverse age-related changes such as cataracts, cardiovascular disease, cancer; cataracts may develop as early as the 20s, with average age of death at 45-50 years.

The International Longevity Center - USA, Ltd. (ILC-USA) is a not-for-profit, non-partisan research and education organization whose mission is to help societies address longevity and population aging in positive and productive ways and highlight older people's productivity and contributions to their families and society as a whole.

The organization is part of a multinational research and education consortium, which includes centers in the U.S., Japan, Great Britain, France, and the Dominican Republic. These centers work both autonomously and collaboratively to study how greater life expectancy and increased proportions of older people impact nations around the world.

INTERNATIONAL

LONGEVITY

CENTER - USA, LTD.

60 East 86th Street

New York, NY 10028

Tel: 212.288.1468

Fax: 212.288.3132

www.ilcusa.org

AN AFFILIATE OF THE

MOUNT SINAI SCHOOL OF MEDICINE