

## Toward a New Understanding of Aging

By the year 2050, 21% of the U.S. population will be age 65 or older, according to the Population Reference Bureau. As our bodies age, our ability to defend against environmental insults diminishes, and exposures can accelerate the aging process and trigger or exacerbate disease. Decreased efficiency in the blood-brain barrier and the cardiovascular, pulmonary, immune, musculoskeletal, hepatic, renal, and gastrointestinal systems can alter response to environmental agents, leading to heightened susceptibility to the toxic effects of air pollution, pesticides, and other exogenous threats to health. Age-related physiologic changes may also contribute to the release of stored toxicants in the body such as lead or organochlorines. Further, life-long exposure to low-level toxicants can increase the risk of diseases common in the elderly, such as osteoporosis, hypertension, renal impairment, Parkinson disease (PD), and Alzheimer disease (AD).

Recognizing the urgency of protecting the health of older Americans, in 2002 the U.S. Environmental Protection Agency (EPA) launched the Aging Initiative, an information- and recommendation-gathering effort designed to shape a planned National Agenda for the Environment and the Aging (for more information on this initiative, see "Aging Research: The Future Face of Environmental Health," p. A760 this issue). The NIEHS is one of several organizations actively participating in and contributing to this initiative. At a National Academy of Sciences workshop titled *Differential Susceptibility of Older Persons to Environmental Hazards*, held 5–6 December 2002 to help shape the agenda, deputy director Samuel Wilson was enthusiastic in his support for the effort. "Our institute is very excited that the U.S. EPA has taken on this topic as an initiative," he said. "[The NIEHS has] a substantial interest in aging-related research."

Substantial interest generates substantial investment. Wilson informed the workshop participants that in the areas of neurotoxicology, neurodevelopment, respiratory disease, cancer, and other chronic diseases involving a relationship between

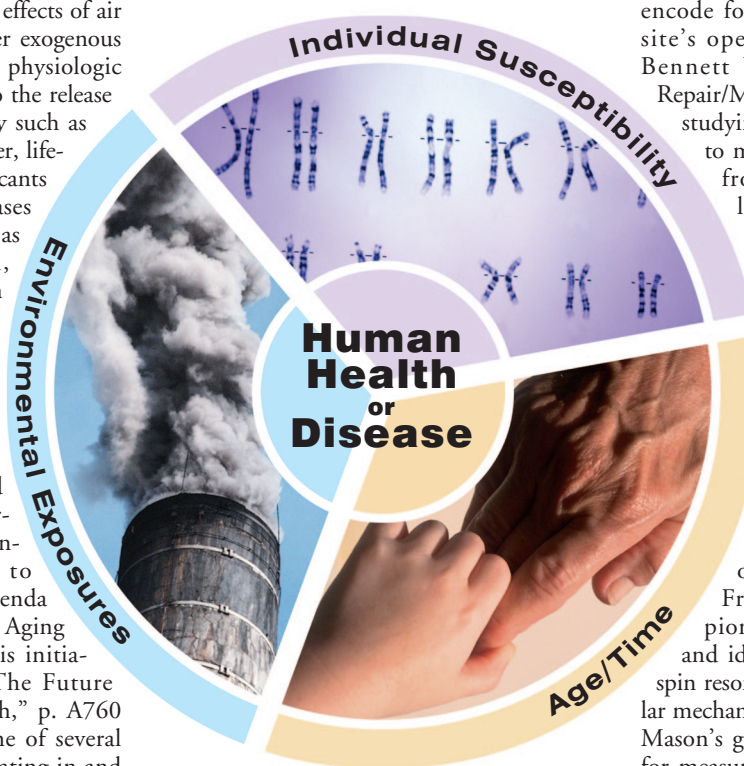
age and environmental exposure, the institute's aging-related research portfolio numbers approximately 50 projects with funding of nearly \$10 million dollars. He added, "There is a substantial research effort in inherited diseases that are associated with advances in aging and with abnormalities in stress response." The research portfolio in that area consists of about 75 projects, with expenditures roughly the same as in the specific age-related portfolio. Together, these investments represent about 5% of the institute's overall research portfolio.

Although there is no NIEHS program specifically dedicated to addressing aging and the environment, the institute's commitment to investigation of the complex

for Cancer Research at the National Cancer Institute) documented the first evidence that aging of cells in culture is an inherently genetically controlled process. Today, two groups within the NIEHS are exploring the so-called mitochondrial theory of aging—the idea that the aging process itself may be due to a lifetime of oxidative damage to proteins, DNA, and lipids in mitochondria, the cellular organelles where food is converted into energy. Senior staff fellow William Copeland and the Mitochondrial Replication Group are addressing the origin of spontaneous mutations in mitochondrial DNA by investigating the accuracy of replication machinery needed to copy the mitochondrial DNA, which consists of 16,569 base pairs that encode for 13 proteins required for the site's operation. Adjunct investigator Bennett Van Houten and the DNA Repair/Mitochondrial Damage Group are studying the consequences of damage to mitochondrial DNA, which aside from aging itself could include links to age-related degenerative diseases such as cancer, AD, PD, and heart disease.

Scientists believe that oxidative stress due to overproduction of free radicals and/or the diminution of protective mechanisms is involved in the pathophysiology of several diseases and aging. Environmental exposures have been shown to contribute to increased generation of such oxidants. Ronald Mason's Free Radical Metabolism Section pioneered a free radical detection and identification technique, electron spin resonance, to investigate the molecular mechanisms involved in oxidative stress. Mason's group is also exploring strategies for measurement of this potentially useful biomarker in humans for clinical diagnosis. NIEHS grantee Qin Chen, an assistant professor of pharmacology and toxicology at The University of Arizona, is also researching the role of oxidative stress in aging. His group has discovered several new molecular targets altered by oxidants, focusing currently on the *p21WAF1/Cip1* gene. Gaining knowledge about these targets and how they are affected by oxidants may advance understanding of aging and its associated diseases.

Acquired genomic changes are believed to play a role in aging and diseases such as cancer. Colleen Jackson-Cook, an associate professor of genetics at Virginia Commonwealth University, is beginning to assess the extent to which individual



interplay between the two runs deeper than grant making—it is an integral element of the stated mission of the NIEHS.

"We really look at things across a life span," says Anne Sassaman, director of the NIEHS Division of Extramural Research and Training. "With some things, we put a lot of emphasis on the early stages of reproduction and development, but a lot of those conditions then continue and may be expressed at older ages as a result of . . . age and time," she says. "So aging is certainly one of the key components of how we look at our research programs."

### Studying the Aging Process

In 1996, an NIEHS research group led by J. Carl Barrett (now director of the Center

differences between twins in acquired chromosomal abnormalities, as well as chromosome-specific telomere lengths, are determined by genetic predisposition or environmental factors. Jackson-Cook says a clearer delineation of the observed variation in those anomalies and the factors contributing to them “will be helpful for designing tests to screen for individual differences in susceptibility to change in the human genome” and possibly predisposition tests for age-related maladies such as cancer, ulcerative colitis, and AD.

NIEHS grantee Howard Hu, a professor of occupational and environmental medicine at the Harvard School of Public Health, has been working with samples and data from the Normative Aging Study, a cohort of adults who have been followed for 40 years. Hu reports that his group’s most recent analyses have shown that subjects with an *ALAD 2* genetic allele experience a greater toxic effect of lead on their kidneys, and that subjects with a higher body burden of lead have a greater risk of a host of disorders, including anxiety, depression, myocardial infarction, hearing loss, and cataracts. Most disturbingly, perhaps, the subjects with higher environmental lead burdens also manifested a steeper decline in cognitive function with aging.

The effects of fine particle exposure on cardiovascular function on susceptible adults are being explored by NIEHS grantees Diane Gold, an assistant professor of environmental health at the Harvard School of Public Health, along with co-principal investigators Doug Dockery and Frank Speizer. A 1999 time-series epidemiologic study involved repeated monitoring of 27 Boston adults aged 60–90 to evaluate whether measures of cardiovascular function varied as air pollution fluctuated. The researchers discovered a significant association between reduced heart rate variability, an important measure of cardiac function, and elevated ambient levels of fine particulate matter and ozone, implying that exposure to these pollutants may decrease vagal tone, resulting in reduced heart rate variability. A similar study conducted the following year by the same group included measures of indoor home exposure and personal exposure to particles. Those results are being prepared for publication.

Three ongoing grants seek explanations for disparities in health and functionality among seniors. Carlos Mendes de Leon, an associate professor at Rush-Presbyterian-St. Luke’s Medical Center in Chicago, is examining the factors behind social and racial inequalities in health among older adults, focusing on disability and decline

in physical function as markers of overall health status. Mendes de Leon and his group hypothesize that older persons of lower socioeconomic status and older blacks experience increased risk of disability due to greater exposure to biologic risk factors and to adverse social and environmental conditions. They are now in the third year of collecting data on disability status in a population of 6,000 subjects living in the Southside area of Chicago, along with detailed assessment of neighborhood conditions.

Brian Schwartz, director of the Division of Occupational and Environmental Health at The John Hopkins University, and colleagues are engaged in the Baltimore Memory Study, a multilevel cohort study of the determinants of cognitive decline in 50- to 70-year-old residents of specific neighborhoods in Baltimore, seeking to quantify and analyze disparities in cognitive functioning by race and ethnicity. Risk factors to be evaluated include environmental agents such as lead and mercury, genetic polymorphisms, health and cardiovascular factors, social and behavioral factors, and neighborhood contextual factors, which include measures such as community socioeconomic status, neighborhood services, physical condition of buildings, and social integration. According to Schwartz, the study “represents a case study in multilevel, multidisciplinary research aimed at integrating knowledge within and across biologic, environmental, social, behavioral, and mathematical sciences.”

Neighborhood context is more of a primary focus in a study being conducted by José Szapocznik, a professor of psychiatry and behavioral sciences at the University of Miami, on the built environment and Hispanic elders’ behavioral health as measured by cognitive functioning, emotional functioning, and individual and social activity. He and his group are investigating how and to what extent the architectural features of the built environment that support social connectedness, such as the presence of a front porch and sidewalks, impact elders’ behavioral health. According to Szapocznik, it is “the first NIH study that has recognized the neighborhood built environment as a potential risk factor.” Having assessed the baseline cognitive functioning of 260 Hispanic elders in Miami’s East Little Havana community, the group will retest the subjects annually for three years. They will then perform longitudinal analysis to examine whether built environment variables influence social behaviors and, as a result, impact the future magnitude and direction of changes in elders’ cognitive and affective functioning.

## Neurodegenerative Diseases

The role of the environment in aging-related neurodegenerative diseases such as PD, AD, and amyotrophic lateral sclerosis (ALS) has long been an area of particular interest to the NIEHS. Recent initiatives have stimulated a great deal of recent research activity in the quest to delineate how genetic susceptibility, environmental exposures, and aging interact to trigger these often devastating disorders.

**PD.** To accelerate the rate of progress in PD research, in August 2002 the NIEHS awarded grants establishing the Collaborative Centers for Parkinson’s Disease Environmental Research (CCPDER) program. CCPDER is a five-year, \$20 million cooperative agreement in which three multidisciplinary centers, in partnership with the NIEHS, share data, ideas, and resources. The centers are located at Emory University, the University of California, Los Angeles, and The Parkinson’s Institute in Sunnyvale, California. Each program is designed to be multidisciplinary, integrating clinical, epidemiologic, and basic science, while actively coordinating efforts and collaborating with colleagues at the other centers and the institute. A steering committee oversees the collaborative activities among the three centers.

The first year of the program has been a building process, strengthening the collaborative relationships between groups within each of the centers and beginning to build effective partnerships between centers. NIEHS CCPDER science administrator Cindy Lawler says the progress is encouraging: “Our investigators are enthusiastic about the potential benefits of working together as a network of centers. By sharing with each other findings that are emerging, center investigators can more quickly react to new findings, including making adjustments in each of their research programs to reinforce or extend the findings of their colleagues.”

The objectives of this effort to jumpstart scientific progress on the world’s second most common neurodegenerative disorder are ambitious. “This program was built on the premise that PD reflects an interaction between genetic susceptibility and environmental factors,” says Lawler. “So the goal of the program is to identify and understand these gene–environment interactions, and then rapidly translate that knowledge into the public health domain. This translation could involve identification of new targets for drug therapy or recommendations for reducing exposures to particular environmental agents.” As a first step toward translation,

the CCPDER is developing a website that will include information describing each of the CCPDER projects and their potential relevance to people with PD.

NIEHS-supported research into PD extends well beyond the CCPDER program. For example, Harvey Checkoway, a professor of environmental and occupational health sciences at the University of Washington, is pursuing a study of gene-environment interactions in PD, with an emphasis on exposures to industrial solvents, heavy metals, and pesticides, along with identification of genotypic risk factors. The hypothesis of PD pathogenesis underlying his group's investigation is that chemicals that provoke oxidative stress reactions destroy dopaminergic neurons preferentially among persons with genetically determined susceptibilities. Checkoway says, "Our study findings to date . . . are suggestive of potentially important relations with dietary iron and manganese."

Deborah Cory-Slechta, director of the Environmental and Occupational Health Sciences Institute in Piscataway, New Jersey, has shown that exposure to a combination of pesticides—the fungicide maneb and the herbicide paraquat—produces a PD-like condition, or PD phenotype, in an animal model. Exploring the question of what other risk factors might modulate this PD phenotype, she has uncovered several potentially important associations.

First, age appears to be a significant risk factor. "We took mice that were six weeks, five months, and eighteen months of age, and exposed them to this pesticide mixture, and got marked effects, particularly in the oldest animals," says Cory-Slechta. "There clearly is an aging component; aging can enhance the effects of these exposures." Other experiments have shown that genetic predisposition can modulate the effects of the pesticide mixture, and that developmental exposures can lead to manifestation of the PD phenotype later in life. "Not only that, but if you take a subset of those animals and challenge them again as adults, they show a very dramatic PD phenotype," she says. Her work could have profound implications for risk assessment questions regarding cumulative neurotoxicity across the life span, sequential exposures, and aging.

**AD.** Other grantees are investigating environmental factors that are strongly suspected to be involved in the pathogenesis of AD, another typically aging-related neurodegenerative disorder. Exposure to aluminum is falling under increasing scrutiny as a risk factor. Stephen Bondy, a

professor of community and environmental medicine at the University of California, Irvine, is examining the theory that aluminum, although it has no intrinsic pro-oxidant properties, may enhance the potential of transition metals such as iron to enhance free radical generation in nerve tissue. He reports that "our experimental findings strengthen the possibility that a prolonged exposure to relatively low levels of aluminum may be neurotoxic. The kinds of deficits that might be expected are not spectacular, but are likely to involve subtle promotion of age-related neurological disease."

Domenico Praticò, a research assistant professor of pharmacology at the University of Pennsylvania, has been investigating the effects of dietary aluminum on amyloidosis, the formation of AD-like plaque deposits, in the brains of transgenic mice. His work, which has previously shown that oxidative stress precedes the onset of amyloid plaques, will elucidate the role of aluminum as a modulator of brain oxidative damage, and will examine the hypothesis that vitamin E, a dietary antioxidant, could delay the onset of amyloid plaque deposition in the animal model.

The potential relevance of mitochondrial dysfunction caused by oxidative damage in the pathophysiology of AD and other neurodegenerative diseases is being investigated by Yeong-Renn Chen, an assistant professor of medicine at The Ohio State University's Davis Heart and Lung Research Institute. He is concentrating on identifying the molecular mechanisms involved when an environmental insult causes defects in two specific mitochondrial enzymes, mitochondrial terminal enzyme and mitochondrial cytochrome *c*.

Sterling Sudweeks, an assistant professor of physiology and developmental biology at Brigham Young University, is attempting to characterize a group of receptors found in the hippocampus (an area in the brain associated with learning and memory) suspected of playing a role in the development of dementia, one of the tragic hallmarks of AD. Neuronal nicotinic acetylcholine receptors (nAChRs), the physiologic site of action in the brain for nicotine, are also crucial to hippocampal activity. They come in many possible subtypes, and their expression can be influenced by environmental exposures.  $\beta$ -amyloid protein, which forms deposits in the brains of AD patients, has been shown to bind to and interfere with synaptic signaling through neuronal nAChRs. Sudweeks hopes that characterizing the subtypes of neuronal nAChRs will shed light on which tend to bind with

$\beta$ -amyloid. This knowledge will help shed light on how environmental exposures can affect individual susceptibility to AD, and ultimately could help identify targets for therapeutic intervention.

### Prostate Cancer

Of course, not all environmental exposures are harmful. In fact, some seem to impart a protective effect against other sources of damage. That's the core of the theory behind senior scientist Coral Lamartiniere's study of prostate cancer at the University of Alabama at Birmingham. His group has discovered that dietary exposure to genistein (a phytoestrogen component of soy) starting at puberty suppressed the development of spontaneous prostate cancer in transgenic mice bred to be highly susceptible to the disease. That laboratory observation correlates with other findings that Asian men who consume a traditional diet high in soy products have a low incidence of prostate cancer.

Lamartiniere hypothesizes that this reduced susceptibility to prostate cancer is dependent on a process called "imprinting." In imprinting, the consumption of genistein at a particular time of development—in transgenic mice this time was before or at the onset of puberty—determines the biochemical blueprint of how the prostate will respond later in life to hormone and growth factor stimuli, which can trigger cancer.

Imprinting of the prostate can apparently also render an individual more susceptible to prostate cancer in his adult years. Gail Prins, an associate professor in the University of Illinois at Chicago Department of Urology, has shown that brief exposure of rodents to high doses of natural or synthetic estrogens during development results in permanent alterations in growth and differentiation of the prostate gland. This estrogen imprinting is associated with prostatic lesions and cancer later in life. Estrogen imprinting could sensitize males to later estrogen exposures, increasing susceptibility to estrogen-induced prostatic tumors. Because estrogen levels rise in the aging male, this "two-hit" scenario could help explain the high incidence of prostate cancer in older men. Prins's group is exploring this scenario in animal models, and investigating the genetic basis of the suspected estrogen imprinting.

### Osteoporosis

J. Edward Puzas, the Donald and Mary Clark Professor of Orthopedics at the University of Rochester, directs a program

in osteoporosis comprising four individual projects, each with its own principal investigator. The unifying hypothesis for the program is that lead adversely affects skeletal metabolism in children and adults to the point that it is a causative factor in bone diseases, especially osteoporosis. The program aims to integrate basic science findings on the effects of lead on the skeleton with the clinical entities of osteoporosis and osteoporotic fractures. The group's preliminary data in both *in vitro* and *in vivo* models suggest, Puzas says, that a significant portion of people with osteoporosis may have the disease due to lead exposure, and that lead in the skeleton will prevent normal healing of fractures.

One of the team's projects is intended to translate knowledge gained from the basic research projects into clinical diagnostic and therapeutic trials. They plan to develop a clinical therapeutic paradigm that will take bone lead burden into account as an important factor in the treatment of osteoporosis.

Lead exposure also figures prominently in a study being conducted by Susan Korrick, a lecturer in occupational health at Brigham and Women's Hospital in Boston. Her objective is to study the effects of environmental lead exposures and allelic variants of the vitamin D receptor on the development of osteoporosis in middle-aged women. Preliminary results support the hypothesis that increased bone lead is associated with prospective decreases in bone mineral density and increased bone turnover activity, both of which are risk factors for osteoporosis. Korrick believes that polymorphisms in the vitamin D receptor genotype may be responsible for increased susceptibility to the disease, and that lead exposure may be involved in triggering or accelerating the skeletal changes associated with osteoporosis.

The above compendium represents just a small fraction of the aging-related environmental health research taking place both within the NIEHS and with its support. Wilson was, if anything, understating the case when he told the December workshop participants that "certainly this is an area that we at NIEHS have been interested in for some time."

Scientists may never discover a way to halt or reverse the aging process. But with the breadth and depth of aging-related research going on at the NIEHS and elsewhere, it seems likely that substantial progress can be expected in the quest to prevent and treat diseases associated with aging, and to ensure that the environmental contribution to aging is restricted to the normal and the natural. —**Ernie Hood**

## Headliners

NIEHS-Supported Research

## Reproductive Toxicants



### DDT and DDE: Effect on Second Generation Time to Pregnancy

Cohn BA, Cirillo PM, Wolff MS, Schwingl PJ, Cohen RD, Sholtz RI, Ferrara A, Christianson RE, van den Berg BJ, Siiteri PK. 2003. DDT and DDE exposure in mothers and time to pregnancy in daughters. *Lancet* 361:2205–2206.

Reproductive tract anomalies have been seen in daughters of women who took the potent estrogenic chemical diethylstilbestrol during pregnancy to prevent morning sickness. Knowing this, NIEHS grantee Barbara Cohn at the Public Health Institute in Berkeley, California, and colleagues decided to investigate whether *in utero* exposure to weakly estrogenic chemicals, including the insecticide DDT and its metabolites, might produce similar adverse reproductive effects.

The World Health Organization estimates that during the period of DDT's use approximately 25 million lives were saved, predominantly from malaria and typhus. However, many species of insects developed resistance to DDT, it proved to be highly toxic toward fish, and it was responsible for the near-extinction of several bird species because of its interference with the formation of egg shells. For these reasons and because of its environmental persistence, the use of DDT was banned in the United States in 1972. However, DDT is still used in some parts of the world.

In mammals, DDT and its major metabolite, DDE, persist in the body and are stored in fat tissue. DDT is known to have weak estrogenic activity, and DDE has considerable antiandrogenic activity. They can cross the placenta, potentially interfering with fetal development.

To further investigate possible effects on the human reproductive system, Cohn's team measured DDT and DDE concentrations in maternal serum samples collected during 1960–1963. The samples were collected within 1–3 days of delivery from women enrolled in the Kaiser Permanente health plan who were participating in the Child Health and Development Studies. The researchers then compared these levels to the time until pregnancy (measured by survey) for 289 daughters of these women.

There was a clear association between increased DDT concentrations in maternal blood and a decreased chance of pregnancy in the daughters. For every 10 milligrams per liter of DDT in maternal serum, the probability of pregnancy dropped 32%. However, quite unexpectedly, the chance of pregnancy increased 16% with each increase of 10 milligrams per liter of DDE.

The opposing effects of DDT and DDE may explain why large changes in reproductive performance overall have not been noticed in humans since the introduction of DDT. Although the decreased fertility associated with *in utero* exposure to DDT remains unexplained, the authors speculate that the "antiandrogenic effects of DDE may mitigate harmful androgenic effects on the ovary during gestation and early life."

This study, the first to link DDT exposure *in utero* to human reproductive problems some 30 years later, demonstrates the long delay from exposure to noticeable effect. The findings support the establishment of new long-term human studies that can monitor the effects of environmental exposures on reproduction, as well as continued support of existing studies where multigenerational follow-up is in progress. —**Jerry Phelps**