

## STORIES OF DISCOVERY

### **Preventing Strokes**

Strokes are the third leading cause of death in the U.S., ranking below only heart disease and cancer, and the estimated 4.4 million stroke survivors<sup>1</sup> often suffer serious, long-term disability. Because the likelihood of a stroke – sometimes called a “brain attack” – increases with age, and the American population is aging, the number of strokes is increasing. However, without the remarkable progress in stroke prevention, which reflects the sustained efforts of private organizations, NIH, and other government agencies, the toll of stroke would be dramatically worse. The U.S. Centers for Disease Control and Prevention estimates that the age-standardized stroke death rate declined by 70 percent for the U.S. population from 1950 to 1996<sup>2</sup>, and the American Heart Association tally notes a 15 percent decline just from 1988 to 1998.

NIH research led to the first acute treatment proven to improve the outcome from ischemic stroke – tissue plasminogen activator or t-PA, and efforts are underway to develop even better interventions to promote recovery. Yet, most of the reduced death rate to date comes from research on stroke prevention, and NIH has contributed substantially to this growing body of knowledge.

Over the past two decades, advances in stroke research have taught us that there is no “one size fits all” approach to preventing stroke. Millions of Americans live with a variety of risk factors – heart irregularities, hypertension, narrowed arteries, diabetes, and others. In addition, women and minorities, as well as people living in specific geographic regions, have unique stroke risks that must be addressed independently from other risk factors.

The large Stroke Prevention in Atrial Fibrillation (SPAF) studies of the 1980s and 1990s illustrate how medical management has contributed to stroke prevention. For many years, aspirin and warfarin – two anti-thrombotic therapies with different safety profiles and monitoring requirements – were used to prevent stroke in patients with atrial fibrillation, a common disorder of irregular heart rate and rhythm, and a significant stroke risk factor. However, use of these agents was based on little hard scientific evidence. The SPAF trials indicated that both aspirin and warfarin are effective and have a place in the prevention armamentarium, but that each drug has a better risk/benefit ratio for a different group of patients. Other studies, such as the Warfarin Antiplatelet Recurrent Stroke Study, the Vitamin Intervention for Stroke Prevention study, the African-American Antiplatelet Stroke Prevention Study, and the Women’s Estrogen

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<sup>1</sup> *Stroke*, Jan. 2001 32:280-299.

<sup>2</sup> *MMWR Weekly* 48:649-56 1999.

for Stroke Trial, build on these earlier findings, and continue to add to our knowledge about medical interventions that can affect the incidence of stroke in different at-risk groups.

The NIH has also supported several major studies in the surgical prevention of stroke. This work has particular significance for people with carotid artery stenosis, a narrowing of the major blood vessels that supply the brain. One definitive study in the late 1970s examined a procedure called extracranial/intracranial (EC/IC) bypass. EC/IC bypass had been used for several years as a means to restore blood flow to the brain, though not always successfully. This NIH-funded study of the procedure's effectiveness found that the data did not support its continued use in medical practice. Although these findings were negative, they were of significant benefit to patients, who could avoid the risks of this surgery, and to researchers, who used this information to redirect their attention to other promising approaches. As a result, investigators explored an alternative strategy to the bypass surgery, called carotid endarterectomy, which involves the removal of fatty deposits in the carotid arteries. This approach was shown to have substantial benefit, in both the Asymptomatic Carotid Atherosclerosis Study (ACAS), and the North American Symptomatic Carotid Endarterectomy Trial (NASCET), for people who meet certain criteria. As with other prevention strategies, NIH continues to evaluate surgical interventions, particularly as new techniques are developed.

Researchers are also continuing to examine gaps in the field of stroke prevention that might benefit from controlled clinical study. Recently, NIH-funded researchers evaluated the risk of stroke after a transient ischemic attack (TIA), or "mini-stroke." The symptoms of TIAs pass quickly, usually within a day, and are often ignored. After following 1,700 people with a TIA, the study found that these episodes warn of a dramatically increased likelihood of experiencing a stroke within a 90-day period. Other risk factors, such as advanced age, other health conditions, and severity of the TIA, also helped to predict stroke risk, and may be useful in determining whether patients should be hospitalized immediately and/or receive preventive interventions following a TIA.

The incremental nature of progress in stroke prevention has confirmed that there is no easy route to success. The broad portfolio of NIH research on stroke offers a glimpse of what the future might bring – from the possibility of vaccines, to genetic tests to tailored preventive measures for each individual, to studies that may link infections or inflammation within blood vessels to stroke. The NIH five-year plan for stroke research and its planning efforts targeting health disparities in stroke will guide these activities to produce continued advances in stroke prevention.

NIH is also strongly committed to expanding its programs to educate clinicians and the public about important research findings. NIH was a key participant in organizing The Brain Attack Coalition – a group of professional, voluntary and governmental entities dedicated to reducing stroke occurrence, disabilities and death – and the Coalition's website is maintained by NIH staff. In addition, in May 2001, NIH launched a national public education campaign, "*Know Stroke: Know the Signs. Act in Time.*" Each year, only a fraction of stroke patients arrive at the hospital in time to receive the only proven acute treatment that makes the difference between

disability and full recovery in ischemic stroke, the “clot buster” t-PA. This campaign is designed to help people recognize the symptoms of stroke and appreciate their urgency, in order to obtain medical help in time.

The gains from stroke prevention research each year may be incremental in their effect on national statistics, but even a small drop in the stroke rate means a dramatic difference for many people and their families, and over time the advances in stroke prevention research are having a major impact on the Nation’s health.

## The Ominous Link between Obesity and Type 2 Diabetes

An epidemic of obesity and type 2 diabetes is striking the U.S., according to epidemiologic studies. Type 2 diabetes is a devastating illness already afflicting more than 16 million Americans, and uncontrolled diabetes leads to serious complications including blindness, kidney failure, lower limb amputations, and heart disease. Although genetic factors may predispose a person to develop diabetes or to be overweight, our genes could not possibly have changed quickly enough to account for the rapid increase in prevalence of obesity and type 2 diabetes. In light of this observation, some environmental factor is probably responsible for the increase. Research indicates that the obesity problem, in a nutshell, is due to the fact that Americans eat too much and exercise too little. But how is the increase in obesity related to the increase in type 2 diabetes? For a long time, researchers have known that persons who are overweight and/or obese are far more likely to develop type 2 diabetes. In fact, 80 percent of patients with type 2 diabetes are overweight or obese. However, the underlying biological reason for the connection between these two health problems has been difficult to pin down.

Type 2 diabetes develops through a multi-stage process. First, the body is unable to use insulin effectively, a condition known as insulin resistance. Insulin is produced by the beta cells of the pancreas, and it causes fat, liver, and muscle cells to store sugar found in the blood. In patients with insulin resistance, research has shown that the beta cells compensate by working harder to make more insulin. For awhile, blood sugar levels are relatively normal because of the increased insulin production. Eventually, however, the beta cells become exhausted and are unable to produce enough insulin to overcome insulin resistance. When this occurs, patients develop a condition known as impaired glucose tolerance, or IGT. In individuals with IGT, blood sugar levels are higher than normal but not yet diabetic. Left untreated, IGT frequently progresses to become full-blown type 2 diabetes. Unfortunately, neither insulin resistance nor IGT has any outward symptoms. Millions of Americans have insulin resistance and IGT but do not know. Although the condition is silent, they are at high risk of developing diabetes over the next few years and are also already at increased risk of heart disease.

How does obesity give rise to diabetes? Clues to the ominous link between obesity and diabetes are being discovered in unexpected places. To the surprise of scientists, fat is not a passive storehouse for energy. Rather it is a metabolically active tissue that senses changes in energy availability and signals the brain to regulate feeding behavior as well as other tissues involved in fuel metabolism. The molecular mechanisms underlying this communication are rapidly being unraveled. Fat cells make and release signaling proteins, called hormones, into the blood in response to energy demands and feeding behavior. These proteins made in fat cells may be the key linking weight gain (and corresponding increase in the number and size of fat cells) and development of type 2 diabetes. Seen from this perspective, it is reasonable to suggest that if you have too much fat, you make too much of a certain hormone, and this causes diabetes. In reality, however, the connection is not so straightforward. Careful research is uncovering many novel signaling proteins produced in fat cells. We now know a complex balance of fat cell hormones is involved in regulating the body's blood sugar levels.

After a meal, fat cells release an appetite-regulating protein called leptin; this hormone signals the appetite-control center in the brain to stop eating. Inappropriate levels of leptin, or the molecules in the brain that respond to leptin, could contribute to overactive appetite and resulting obesity due to a communication breakdown between the fat cells and the brain. The existence of this type of link is substantiated by studies showing that mice lacking the gene for leptin overeat and become extremely obese. When treated with leptin, the obese mice lose weight; however, administration of leptin as a treatment for common forms of human obesity has not been effective, suggesting that other factors are at work.

Another fat-cell protein, resistin, is so-named because too much resistin is thought to cause liver and muscle cells to become insulin resistant. Fat cells of obese patients and diabetic patients do not appropriately regulate resistin production in response to food intake. Ongoing studies are describing the structure and signaling mechanisms used by resistin. This hormone and the proteins that respond to it may be promising targets for development of medicines to treat or prevent diabetes.

Another recently identified fat-cell protein important for energy regulation is Acrp30, also called adiponectin. In mice eating a high-fat, high sugar diet, treatment with adiponectin causes muscle cells to burn more energy and can reduce body weight despite a continued high calorie diet. In humans, adipocytes release adiponectin into the bloodstream, where it influences energy regulation by signaling between fat and muscle cells. Studies suggest that overweight patients and diabetic patients may have impaired adiponectin production. Adiponectin causes liver and muscle cells to be more sensitive to insulin, and insufficient adiponectin could cause insulin resistance. Mouse models of both obesity and type 2 diabetes became less insulin-resistant with treatment increasing adiponectin within physiological levels. In addition, scientists discovered that there is a linkage with diabetes susceptibility near the site in the human chromosome where the adiponectin gene is located.

Regulation of leptin, resistin, adiponectin, and other fat-cell hormones is influenced by body weight and fat mass. These newly discovered fat-cell hormones may be among the missing links between obesity and type 2 diabetes. Already, anti-diabetic drugs called TZDs (thiazolidinediones), that regulate gene expression in fat cells, are used to correct blood sugar and lessen insulin resistance. As we learn more about the specific fat-cell proteins involved in metabolic regulation, drug therapies can be developed to more specifically target the balance of fat-cell-secreted proteins important for blood sugar regulation.

At the same time that basic scientists are learning about what causes obesity and diabetes, clinical researchers are developing practical measures to combat their increase. An exciting new study has added new weapons to our arsenal for battling the epidemic of obesity and type 2 diabetes. Results from a major clinical trial demonstrate that patients at risk of developing type 2 diabetes can prevent disease onset and improve their blood sugar through modest improvements in diet and exercise. The results are particularly important to minorities, who made up 45 percent of study participants and are at increased risk of developing diabetes. This study, called the Diabetes Prevention Program, or DPP, identified overweight individuals at risk

for developing type 2 diabetes because they suffered from the Impaired Glucose Tolerance described above. All patients in the study were counseled about the benefits of reduced-fat diet, weight loss, and exercise. Following this standard counseling, patients were assigned to one of three groups: intensive lifestyle intervention, medication or placebo control. The intensive lifestyle intervention was most effective: patients in this group were 58 percent less likely to develop diabetes than those in the control group, which received only the standard counseling and a placebo drug. The intensive lifestyle intervention had a goal for each patient to reduce body weight by seven percent and to stay active through a minimum of 30 minutes of exercise at least five times per week. Patients assigned to the medication group, and treated with the anti-diabetic drug metformin, were 31 percent less likely to develop diabetes than the control group. Nearly one-third of patients in the control group, who received a placebo drug, developed diabetes. The intensive lifestyle intervention was highly effective for all ages, genders, and racial/ethnic groups in the study, and it was the most effective treatment for preventing type 2 diabetes. Metformin, while less effective overall at reducing the risk of diabetes, was also effective in both genders in all the racial and ethnic groups; (African-Americans, Hispanic Americans, Asian Americans and American Indians were all well represented). In contrast to lifestyle, which was highly effective for all age and weight groups and particularly effective in older patients, metformin's effect was limited to certain age and weight groups. Metformin treatment was most effective for younger patients and those who were extremely obese, and was not effective in people over 60. The lifestyle intervention appears to exert its major effect through a reduction in weight averaging about 15 pounds after the first year and 10 pounds after three years of participation in the study. Metformin caused a more modest weight loss and appears to act through additional mechanisms to prevent diabetes. This landmark study showed that with instruction and encouragement patients at high risk for diabetes could be successful in improving their diet and activity and that these relatively modest changes had a major impact in reducing the onset of diabetes.

## **The Maternal Side of Mother to Child HIV Transmission**

From the first reports of HIV transmission from mother to child during pregnancy, delivery and breast feeding in the 1980s, scientists have been working to try to understand the ways it is transmitted, at what levels, and what factors increase or decrease the risk of transmission. Attention is focused on understanding this route of transmission and in designing interventions to reduce HIV infection in newborns born to HIV-infected women. The end point in most studies was HIV infection in the newborn. Although some concern was voiced among maternal and child health advocates about the health of the mother and how this affects the survival of her children, little attention was made by AIDS scientists to outcomes beyond infant HIV infection.

Initially, research focused on studies to estimate the percentage of pregnant women and the rates of HIV transmission among their newborns so that the magnitude of the problem could be estimated. These studies found that the percentage of pregnant women who are HIV infected range from less than 5 percent in most countries in Latin America and Asia to over 20 percent in some countries in eastern and southern Africa. Studies among the newborns born to infected women revealed that the rates of transmission varied from 15 percent to 45 percent with populations in which the majority of the women breastfeed having higher rates (25-45 percent). Further studies confirmed that the breast milk of infected women did contain the HIV virus, and that HIV infection was transmitted through breastfeeding. This finding was very disturbing as breastfeeding has long been recognized as crucial to child survival in many resource-poor countries and has been promoted for years by maternal and child health advocates and policy makers in governments. As a result, scientists focused on studies to better understand HIV transmission through breastfeeding.

Additional studies revealed that the highest rate of infection for newborns seems to occur during labor and delivery (10-20 percent) and ranges from 5-10 percent during pregnancy and during breastfeeding. The proportion of infections that occur due to breastfeeding varied, depending on breastfeeding practices and the level of virus contained in the breast milk. However, these studies were primarily observational studies of populations in which almost all the women breast fed or in which almost all the women formula fed. From this type of study, it was impossible to determine if unknown factors that were associated with the method of feeding chosen by the women could be influencing the rates of HIV transmission. The best way to control for these unknown factors is to conduct a study in which women are randomly assigned to how they would feed their newborns. This would result in a study in which half the women breastfed and half did not, and in which the decision about how to feed was not made by the woman.

Such a study was conducted in Kenya from 1992-1998. This study found that babies born to mothers who were randomized to breast feed were more likely to be HIV infected at 24 months (36.7 percent) than those babies born to mothers who were randomized to formula feed (20.5 percent). Hence the rate of transmission through breastfeeding was estimated to be about 16 percent; most (75 percent) of the infections occurred in first 6 months of breastfeeding. The finding that the mortality of the newborns in the two groups did not differ was somewhat re-

assuring, as many breastfeeding advocates had voiced concerns that formula feeding would put these children at risk of death from other infections and diarrhea.

The investigators who conducted the breastfeeding study in Kenya, were able to use their data to look at two additional outcomes of interest: mortality in the mothers in the two groups (breast feed and formula feed) and the impact of the mother's death on the outcome of her newborn. Their findings startled many in the scientific community. Infected mothers who breastfed were three times more likely to die in the two years following delivery than the mothers who did not breastfeed. In addition, infants whose mothers died in this period were eight times more likely to die than infants whose mothers survived, even taking their HIV infection status into account. The death of mothers in resource poor countries is a well-known risk factor for subsequent death among her children, even before the AIDS epidemic.

Although the reason for the increased mortality in the breastfeeding mothers remains unclear, this finding stimulated other AIDS researchers to look more closely at their data for maternal outcomes. Researchers who conducted an observational study in South Africa that compared different types of feeding practice patterns do not find increased mortality among their breastfeeding mothers. However, the Kenya results, suggesting that breastfeeding by HIV-1 infected women may result in adverse outcomes for the mothers and quantifying the eight-fold risk of death among newborns whose mothers die, have re-directed researchers to look beyond infant HIV infection in their studies of mother to child transmission of HIV.

Because many HIV infected women in resource-poor settings choose to breastfeed for economic and cultural reasons, these findings highlight the need to better understand how breast feeding adversely affects the mother and what can be done to reduce these adverse effects. In addition to interventions to reduce mother to child HIV transmission, interventions for the HIV-infected mother after delivery so that she survives as long as possible are critical to increase survival among infants born to these mothers. The World Health Organization issued a statement in response to the publication of the Kenyan and South African studies. The statement called for more attention to the support and care of HIV infected mothers, further expansion of HIV testing for women, and increased access for pregnant women to programs that prevent mother to child transmission and provide care and support for them.

## Understanding What Goes Wrong in PTSD: Pathways to Prevention

People with post-traumatic stress disorder (PTSD) seem haunted by the past, unable to escape the harrowing grip of a traumatic event. It pursues them – for months, years, or even a lifetime – through flashbacks, memories, nightmares, or frightening thoughts that can make routine activities at work, school, home, and with friends nearly impossible. They are often anxious and hypervigilant, trying to avoid potential reminders of the trauma. At the same time, PTSD can also bring emotional numbness, sleep disturbances, depression, irritability, outbursts of anger, and feelings of intense guilt.

Within the past few decades, PTSD, once seen as a hard-to-treat psychological disorder of “shell-shocked” veterans, has taken on a new identity. It is now seen as an increasingly treatable – and potentially preventable – psychobiological disorder of children as well as adults. And it can follow a variety of terrifying events in addition to military combat, including violent personal assaults, disasters, and accidents. As research has advanced, PTSD has become a major focus for understanding how a broad range of traumatic experiences can affect several biological systems important in development and healthy functioning.

PTSD research progressed rapidly during the past decade. Early research established that traumatic stress reactions – especially PTSD – could lead to serious psychiatric symptoms, tended to be chronic in many traumatically stressed victims, and were among the most prevalent of mental health problems. Recent studies have focused more on the sources of very diverse symptoms in traumatized populations and on how symptoms remit or persist over time. They have been very helpful in identifying risk and protective factors for traumatic stress responses and have offered clues for developing new interventions. Child and family studies of disasters and traumatic events have clarified age-specific psychological, social, and behavioral responses to traumatic stress, as well as approaches to intervention.

Despite these advances, predicting which traumatized individuals will go on to develop PTSD remains a challenge, as does finding effective treatments for all who suffer from this debilitating disorder. Research has shown that a number of symptoms are widespread among disaster survivors and are readily treated with appropriate short-term supportive therapy and reassurance, allowing many traumatized people to resume normal and healthy lives. This research also indicates that traumatized people with avoidance and numbing symptoms are more likely to develop PTSD, which requires ongoing treatment. Strong research evidence suggests that cognitive and behavioral therapies, in combination with selected medications, can be effective in alleviating PTSD symptoms and accompanying depression in many people with PTSD. But for those with persistent or chronic PTSD, treatments are often only partially successful, underscoring the importance of developing preventive interventions that can decrease the chances of developing chronic PTSD.

To aid those at risk for PTSD, researchers are seeking clues in basic research on cognitive processing, arousal, and memory. A particularly promising body of research links the psychological aspects of traumatic stress reactions to the many neurobiologic systems activated

under stress that allow individuals to assess and respond appropriately to potential dangers. These converging lines of research include animal studies of the biological and behavioral effects of unpredictable and uncontrollable stress, descriptive studies of behavioral and biological dysregulation in humans, and clinical studies examining how the severity of PTSD symptoms is linked to neural functioning, processing of memories, and interpretations of trauma. Taken together, these studies have sharpened understanding of the symptoms and course of traumatic stress reactions.

Traumatic events particularly affect the sympathetic nervous system (SNS) as well as the hypothalamic-pituitary-adrenal (HPA) axis, which mediates the biologic stress response. The SNS plays a central role in the organism's fight/flight response by increasing blood flow to muscles and vital organs and mobilizing energy for use by large muscle groups. The catecholamines epinephrine and norepinephrine are two key neurotransmitters related to SNS activity, and are released from the medulla of the adrenal gland during periods of high stress. They act upon the brain's hippocampus to aid memory storage, so that emotionally salient or arousing events are more likely to be remembered better and longer than emotionally neutral events. NIH-supported research findings suggest that in some people at risk for PTSD, the SNS over-responds to stress, which is most evident when these individuals are re-stressed. In particular, recent studies have shown that traumatized individuals who have higher heart rates in the emergency room – indicating greater SNS activation – are more likely to develop PTSD later.

The HPA axis plays a restorative role in stress that can also be disrupted by trauma. The hypothalamus releases corticotropin-releasing factor (CRH), leading eventually to the adrenal gland's release of cortisol, which helps to shut down a variety of neurobiologic reactions set in motion by stressful stimuli – including the catecholamine surge. Cortisol levels normally rise in response to stress. However, several studies have found that initial cortisol responses are lower in trauma victims who go on to develop PTSD than in those who do not; further, individuals with PTSD often have lower cortisol levels than normal controls. This may indicate abnormalities in the feedback mechanisms regulating cortisol. One hypothesis developed from NIH-funded research holds that when, in response to trauma, cortisol cannot buffer norepinephrine's effects, the excess of norepinephrine acting on the hippocampus causes traumatic memories to form. In an important study addressing the relationship between catecholamine activation and acquisition of memory, researchers found that persons given propranolol, a drug that blocks adrenergic activation, before being exposed to emotionally disturbing and neutral pictures later recalled less about the disturbing pictures but not the neutral ones. In contrast, persons given a placebo had significantly better memory for the emotional pictures than the neutral ones, suggesting that beta-adrenergic activation is involved in the enhanced memory associated with arousing or emotional experiences. While enhanced memory for arousing or fearful situations may have significance for survival (leaving one less vulnerable to potentially dangerous situations) these memories, in the form of intrusive recollections and nightmares, also may repetitively haunt the trauma survivor long after the event. Moreover, when the traumatic event is relived through intrusive recollections, flashbacks and nightmares, epinephrine and norepinephrine are again released leading to an additional strengthening of the memory and an even greater likelihood of

subsequent intrusive recollections. This process could help explain the progression from subclinical to clinical PTSD seen in patients with delayed onset PTSD.

Based on this knowledge, new research is examining whether chemicals that block abnormal stress responses after a trauma can prevent or reduce the development of PTSD. One NIH-funded study is exploring whether propranolol given within hours of a traumatic event, can prevent the onset of PTSD. This research stems directly from the prior studies of stress and catecholamines. Another ongoing study is comparing the preventive effects of propranolol with another drug, gabapentin, which is usually used to prevent seizures in epilepsy. Gabapentin was chosen to counteract the presumed tendency of repeated episodes of stress to increase activation in brain structures that organize responses to aversive stimuli, such as the amygdala. Finally, new NIH-supported studies are expanding our ability to predict those who may be at risk for PTSD. In one large-scale prospective study, recruits to police, fire, and emergency medical technician units are given a battery of tests over time. On the basis of prior research, the investigator hypothesizes that recruits who are more responsive to aversive stimuli are more likely to develop PTSD after traumatic events at work.

Converging multi-disciplinary studies have already led to the development and testing of new preventive and treatment interventions for PTSD. While much remains to be clarified – such as the exact causes of lowered cortisol levels in people with PTSD and recently reported neuroanatomical differences in people with PTSD – research directions for the future point to exciting pathways of discovery and improved treatment and prevention.

## Parkinson's Disease – Identifying the Environmental Triggers

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's Disease. Most cases begin after the age of 50, and its incidence increases as a function of age. Like most diseases, PD appears to arise from the interaction of three events – an individual's inherited genetic susceptibility, his/her subsequent environmental exposures, and his/her age. Recent studies in twins indicate that genetic susceptibility is the primary determinant for early onset PD, but late onset PD (after age 50), which accounts for the majority of cases, arises from some other factor, possibly of environmental origin.

Important insights into environmental triggers of PD were gained in the early 1980s when a synthetic heroin elicited severe Parkinsonism in addicts who injected it intravenously. The initiating agent was found to be 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), a compound with structural similarities to some herbicides and pesticides. Subsequent case-control studies have found increased incidence of PD associated with pesticide use, rural environments, consumption of well water, exposure to herbicides, and living near industrial plants, printing plants, or quarries.

The defect that is a hallmark of the disease is the loss of brain cells that produce a chemical—dopamine – that helps direct muscle activity. Neurotoxicants that act on this system are obvious candidates for setting the stage for PD. Recent work shows that a multi-neurotoxicant exposure scenario might explain some PD cases.

One researcher was studying the herbicide, paraquat, which acts adversely on the dopamine system. She noticed that it shared an extensive geographical overlap in use with other agricultural chemicals, particularly certain fungicides. When paraquat and the fungicide, maneb, were jointly administered to mice, the combined exposure decreased motor activity, increased dopamine turnover, and reduced other measures of dopamine effect at levels far greater than when the same chemicals were administered singly. The fact that combined exposures, such as would be found in real-world applications, can potentiate the adverse effects on the dopamine system raises important possibilities for multiple environmental risk factors being associated with PD development. This work is complemented by another group of researchers who found that the organic pesticide, rotenone, produced symptoms of PD in rats that were given steady amounts of it in their blood stream.

The possible role of multiple exposures in PD risk has also been demonstrated in human studies. A group of researchers examined groups of people occupationally exposed to heavy metals. They also found that exposures to combinations, rather than single compounds, were associated with increased risk of developing PD. In this case it was mixtures of lead, copper, and iron that posed a greater risk than did exposure to these metals singly.

These studies show that agricultural exposures and certain metals could trigger some PD in some people. Equally important, it might well be exposures to a complex of chemicals, rather than single chemicals, that are critical in many cases of PD. This possibility needs to be furthered examined and should be considered when regulatory standards are set.

## Genetic and Molecular Basis of Longevity

Jeanne Calment of Arles, France is believed to have lived longer than any other person in recorded history. When she died on August 4, 1997, she was 122 years, 5 months, and 14 days old. Life expectancy in the U.S. has risen dramatically in the 20th century, from about 47 years in 1900 to about 73 for men and 79 for women in 1999; however, of the world's current 6 billion inhabitants, perhaps no more than 25 people are more than 110 years old. What factors allowed Jeanne Calment to live such a long life? More than likely, heredity, environment, and lifestyle all have complex roles in determining a long and healthy life. But, is there a maximum human life span beyond which we cannot live no matter how optimal our environment or favorable our genes? And perhaps, most importantly, how can insights into longevity be used to fight age-related diseases and disabilities to ensure a healthy, active, and independent life well into very old age?

Since the 1930s, investigators have consistently found that laboratory rats and mice live up to 30 percent longer than usual when fed a diet that has at least 30 percent fewer calories than they would normally consume, but is nutritionally balanced. This was the first demonstration that the maximum life span of a mammal could be increased. More recent research has found that these animals also appear to be more resistant to age-related diseases including cancer. Other rodent studies have found that caloric restriction may increase resistance of neurons in the brain to dysfunction and death. In fact, caloric restriction appears to delay normal age-related degeneration of a number of physiological systems in rodents.

Studies on the effects of caloric restriction in higher mammals (monkeys) are ongoing. Preliminary results are promising, including greater resistance to diabetes and heart disease in these animals. Yet even if caloric restriction is successful in extending primate life span, it is doubtful that it will ever become an acceptable means for most humans. However, caloric restriction shows that life span can be altered, prompting research into possible mechanisms.

Why calorically restricted animals live longer and have reduced rates of age-related diseases is still unclear. Over the years, scientists have been unraveling pieces of this puzzle by identifying and characterizing genes that modify the life span of various organisms including yeast, fruit flies, worms, and mice to determine which biological pathways are involved in life span extension and to determine if these same pathways may be affected by caloric restriction or other interventions. At least 15 different genetic manipulations have been identified in the past ten years that extend the life span of these organisms. These genetic manipulations pinpoint three metabolic systems: the cellular response to stress, especially oxidative stress; hormonal control; and processes like metabolic rate that are altered by caloric restriction.

**Oxidative stress.** The free radical theory of aging was first proposed in 1956 by chemist Denham Harman. Free radicals are by-products of metabolism that can cause extensive damage to proteins, membranes, and DNA unless they are stopped by antioxidants, particularly superoxide dismutase (SOD), an enzyme produced within the cell. Studies have shown that inserting extra copies of the SOD gene into fruit flies extends their average life span by as much as 30 percent and in one compelling study, giving *C.elegans*, a tiny worm with a very short life span, synthetic forms of antioxidants significantly extended their life. Similar experiments in rodents have either not yet been done or have not extended life span, although there is the

possibility that a longevity mutation in another mouse gene might function through increased resistance to oxidative stress. Interestingly, caloric restriction increases the resistance of organisms, including mice, to stresses like oxidative stress or increased temperature, again suggesting again that there may be a relationship between stress resistance and aging.

**Hormonal control.** A major breakthrough occurred in 1995 when it was discovered that mutations in certain other genes in *C.elegans*, can also substantially extend its normal two to three-week life span. One of these genes, called *daf-2*, controls a special stage in the worm's development called dauer formation, a metabolically slowed, non-aging state that it enters when food is limited or there is overcrowding. Other investigators have detected mutations in similar *daf* genes that increase life span three or even four-fold.

These genes are similar in structure to the mammalian genes for a receptor that binds the hormone, insulin, to cells and for an enzyme involved in causing cellular changes in response to insulin binding, the so called IGF-1 signaling pathway. The similarities suggest that worms also have an IGF-1 like signaling pathway, and that reducing its activity may increase their life span. In the late 1990s, researchers discovered that fruit flies also have such a pathway, and that mutations in the genes for this pathway also extend fruit fly life span.

Around the same time, a paper showed a possible relationship between IGF-1 activity and longevity in mice: dwarf mice have low levels of several hormones, including growth hormone, which normally stimulates production of IGF-1. These mice have low levels of IGF-1 and are also long-lived. A critical paper published this year showed that mutations that stop growth hormone function in mice not only increase life span, but also delay cellular senescence, suggesting an effect on the rate of aging as well as on life span. These results highlight the important influence of hormonal regulation on aging.

**Metabolic rate.** A mutation in a gene affecting metabolism also increases life span in fruit flies. This mutation is found in a protein that carries metabolic products of carbohydrates and fats called dicarboxylic acids into the energy factories of the cell, the mitochondria, where the dicarboxylic acids are converted into chemical energy. The result of the mutation is lowered access of mitochondria to dicarboxylic acid fuels, lowered energy production and increased fruit fly life span. This result may be a clue to one mechanism extending life span by calorie restriction as it is likely that caloric restriction would similarly restrict fuel available to mitochondria for conversion into energy.

**Implications for human aging.** The genes isolated so far are only a few of what scientists think may be dozens, perhaps hundreds, of longevity- and aging-related genes active in many different body pathways. The next big question is whether counterparts in people (human homologs, or orthologs) of the genes found in laboratory animals have similar effects. If they do, these ultimately could yield clues about how genes interact with environmental factors to influence longevity in humans. The outcome of this ongoing exploration of genetic and non-genetic factors affecting life span has been to show that aging is not as immutable as previously supposed, and that we may be eventually able to identify practical ways of extending active and healthy life span in humans.