

Biochemistry and Aging

As important as genes are, they do not act in a vacuum. Everyday metabolic activities – even breathing – expose cells to biochemical substances that can promote random DNA damage and other cellular breakdowns. Of these factors, oxygen radicals and crosslinking of proteins have become focal points of scientific exploration. Gerontologists also are studying other important proteins – heat shock proteins, hormones, and growth factors – that may play a role in aging and longevity. In short, the biochemistry of aging is a rich territory with an expanding frontier.

Oxygen Radicals

Oxygen sustains us. Every cell in the body needs it to survive. Yet, paradoxically, oxygen also wreaks havoc in the body and may be a primary catalyst for much of the damage we associate with aging. This damage occurs as a direct result of how cells metabolize it.

Oxygen is processed within a cell by tiny organelles called mitochondria. Mitochondria convert oxygen and food into adenosine triphosphate (ATP), an energy-releasing molecule that powers most cellular processes. In essence, mitochondria are furnaces, and like all furnaces, they produce potentially harmful by-products. In cells, these

by-products are called oxygen free radicals, also known as reactive oxygen species.

A free radical can be produced from almost any molecule when it loses an electron from one or more of its atoms. In cells, they are commonly created when mitochondria combine oxygen with hydrogen to form water. This transformation releases energy into the cell, but it also can shred electrons from oxygen. When this happens it leaves the oxygen atom—now an unstable oxygen free radical—with one unpaired electron. Because electrons are most stable when they are paired, oxygen free radicals steal mates for their lone electrons from other molecules. These molecules, in turn, become unstable

and combine readily with other molecules.

This process, called oxidation, can spark a chain reaction resulting in a series of products, some of which are actually beneficial. The immune system, for instance, uses free radicals to destroy bacteria and other pathogens. Another oxidizing molecule, called nitric oxide, helps nerve cells in the brain communicate with each other.

Free radicals, however, also can be vandals that cause extensive damage to proteins, membranes, and DNA. Mitochondria are particularly prone to free radical damage. The major source of free radical

This mitochondrion is a cellular powerhouse, continuously generating ATP, an energy-releasing molecule, as well as potentially harmful by-products such as oxygen free radicals. Hundreds of mitochondria exist in every cell. With age, increasing oxidative damage to these organelles may decrease their efficiency.



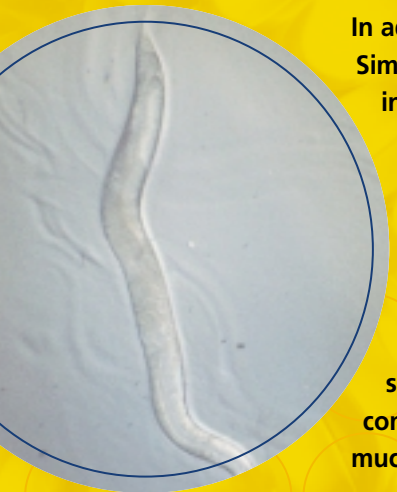
Antioxidants and Aging Nematodes

A worm barely the size of a comma printed on this page may change how investigators think about oxygen radicals, antioxidants, and aging. *C. elegans* nematodes immersed in a liquid containing a potent antioxidant drug lived 44 percent longer than worms not treated with this substance. It was the first time that any drug had extended the lifespan of any multi-cellular organism. The finding lends credence to the hypothesis that oxygen radicals have a significant role in the aging process.

In addition, the international team of investigators, led by Simon Melov, Ph.D., of the Buck Center for Research in Aging in California, restored a normal lifespan to mutant worms that had a mitochondrial defect, which caused increased oxygen radical production and accelerated aging. The worms showed no apparent ill effects from the treatment.

EUK-134, the drug used in the experiment, was a synthetic form of superoxide dismutase (SOD) and catalase, two enzymes that counteract the effects of oxidative stress. Like other antioxidants, such as vitamin E, these compounds convert oxygen radicals to water. But they are much more potent.

While it is only one study, and its results have not been confirmed in other species, this investigation supports the concept that antioxidant defenses may be critical during aging. It also suggests that one day it may be possible to use similar interventions to treat age-related conditions in humans.

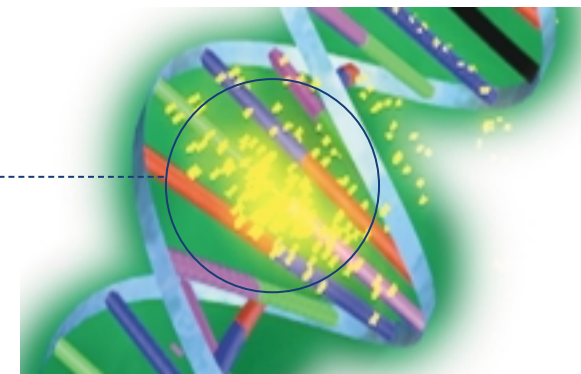


production in the body, they are also one of its prime targets. As the damage mounts, mitochondria become less efficient, progressively generating less ATP and more free radicals. Over time, according to the free radical theory, oxidative damage accumulates in our cells and tissues, triggering many of the bodily changes that occur as we age. Free radicals have been implicated not only in aging but also in degenerative disorders, including cancer, atherosclerosis, cataracts, and neurodegeneration.

But free radicals, which also can be produced by tobacco smoke, sun exposure, and other environmental factors, do not go unchecked. Cells utilize

substances called antioxidants to counteract them. These substances including nutrients—the familiar vitamins C and E—as well as enzymes produced in the cell, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, prevent most oxidative damage. Nonetheless, some free radicals manage to circumvent these defenses and do harm. As a result, cellular repair mechanisms eventually falter and some internal breakdowns are inevitable. These breakdowns can lead to cellular senescence, and eventually may trigger apoptosis, a form of programmed cell death.

Oxygen free radicals, the bright yellow spots in this illustration, can attack and damage DNA, leading to mutations.



Support for the free radical theory, which was first proposed in 1956 by chemist Denham Harman, M.D., comes from studies of antioxidants, particularly SOD. SOD converts an oxygen radical known as superoxide anion into hydrogen peroxide, which can be degraded by an enzyme, called catalase, into oxygen and water. Studies have shown that inserting extra copies of the SOD gene into fruit flies extends their average lifespan by as much as 30 percent.

Other experimental evidence lends support to the free radical hypothesis. For example, higher levels of SOD and catalase have been found in long-lived nematodes. In one compelling study, giving nematodes synthetic forms of these

antioxidants significantly extended their normal lifespan. (See **Antioxidants and Aging Nematodes**, page 19).

The discovery of antioxidants raised hopes that people could retard aging simply by adding them to the diet. So far, studies of antioxidant-laden foods and supplements in humans have yielded little support for this premise. Further research, including large-scale epidemiological studies, might clarify whether dietary antioxidants can help people live longer, healthier lives. For now, however, the effectiveness of dietary antioxidant supplementation remains controversial. In the meantime, gerontologists are investigating other intriguing biochemical processes affected by free radicals, including protein crosslinking.

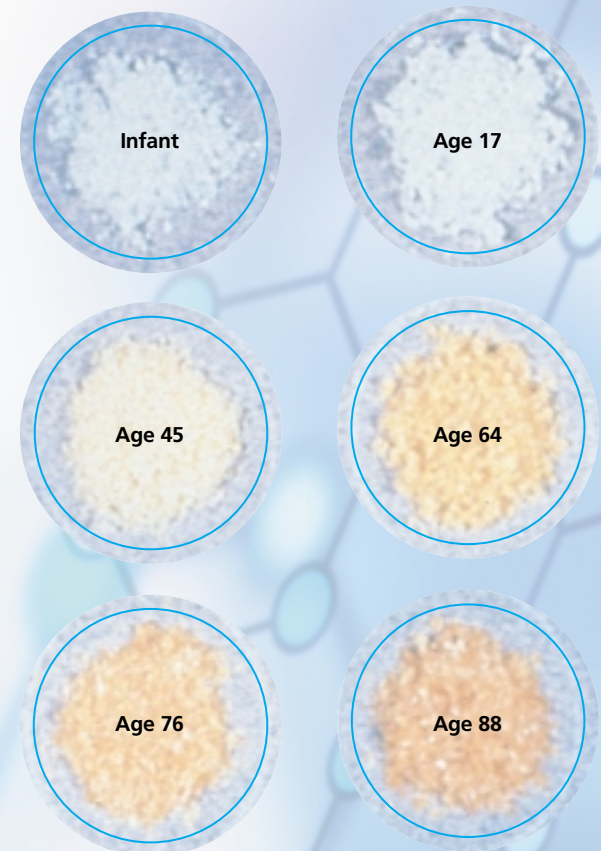
Protein Crosslinking

Blood sugar — glucose — is another suspect in cellular deterioration. In a process called non-enzymatic glycosylation or glycation, glucose molecules attach themselves to proteins, setting in motion a chain of chemical reactions that ends in the proteins binding together or crosslinking, thus altering their biological and structural roles. The process is slow and complex, but cross-linked proteins accumulate with time and eventually disrupt cellular function.

Investigators suspect that glycation and oxidation are interdependent processes since free radicals and crosslinks seem to accelerate the formation of one another.

Protein Crosslinking

Human rib cartilage shows the effects of protein crosslinks, which slowly accumulate through complex pathways and eventually disrupt cellular function. With age, as glucose binds to its proteins, the cartilage turns brown and becomes less supple. This process, called the Maillard reaction, is similar to what happens when bread is toasted.



Research on Sunlight May Help Explain What Happens to Skin as We Age

As anyone who reads beauty magazines knows, sunlight damages skin in ways that seem similar to aging. It's well-established that long-term, sunlight-induced damage causes wrinkles. And in both normal aging and photoaging—the process initiated by sunlight—the skin becomes drier and loses elasticity. Although gerontologists think that the normal or intrinsic aging process is probably not the same as photoaging, there are enough similarities to make this a tantalizing field of study.

The process of photoaging may hold clues to normal aging because many of the same cells are affected. Photoaging, for example, damages collagen and elastin, the two proteins that give skin its elasticity. These proteins decline as we age, along with the fibroblast cells that manufacture them. In addition, the enzymes that break down collagen and elastin increase.

Other changes occur in keratinocytes, upper-layer skin cells that are shed and renewed regularly. In the normal aging process the turnover of keratinocytes slows down and in photoaging they are damaged. Still other skin cells, called melanocytes, are also affected by both processes: they decline with normal aging and are killed in photoaging. (Stopped in their tracks by sunlight, these normally migratory cells show up as freckles in light skin.)

What we don't know yet is exactly how photoaging damages cells. Ultraviolet light can damage DNA and could be the culprit. Free radicals could be involved in some way. Researchers continue to explore these and other factors in the effort to understand photoaging.

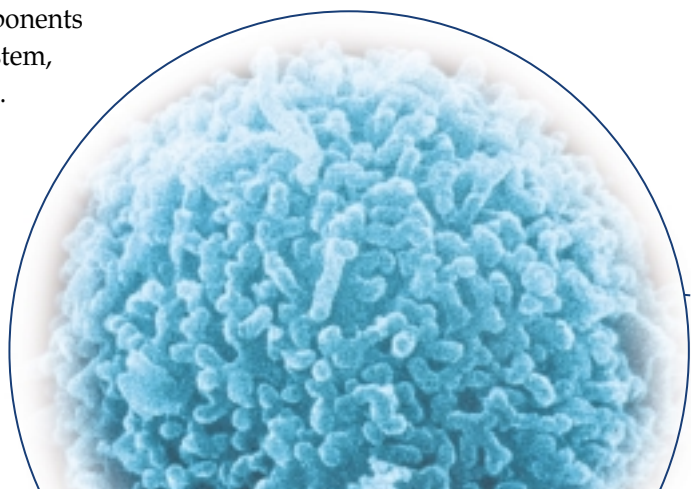
Crosslinks, also known as advanced glycation end products (AGEs), seem to “stiffen” tissues and may cause some of the deterioration associated with aging. Collagen, for instance, the most common protein molecule in our bodies, forms the connective tissue that provides structure and support for organs and joints. When glucose binds with collagen—as it tends to do as we age—this normally supple protein loses much of its flexibility. As a result, lungs, arteries, tendons, and other tissues stiffen and become less efficient. In the circulatory system, AGEs may help trap LDL (the so-called “bad”) cholesterol in artery walls, and thus contribute to the development of atherosclerosis. They also have been linked to clouded lenses (cataracts), reduced

kidney function (nephropathy), and age-related neurological disorders including Alzheimer's disease.

These conditions appear at younger ages in people with diabetes, who have high glucose levels (hyperglycemia). Glycosylated hemoglobin in red blood cells, for instance, is an important marker doctors use to measure hyperglycemia. While the physiological effects of glycosylated hemoglobin are unclear, the disease it helps doctors detect—diabetes—is sometimes considered an accelerated model of aging. Not only do the complications of diabetes mimic the physiologic changes that can accompany old age, but people with this condition have shorter-than-average life expectancies. As a result, much research on cross-linking has focused on its relationship to diabetes as well as aging.

Just as the body has antioxidants to fight free-radical damage, it has other guardians, immune cells called macrophages, which combat glycation. Macrophages with special receptors for AGEs seek out and engulf them. Once AGEs are broken down, they are ejected into the blood stream where they are filtered out by the kidneys and eliminated in urine.

The only apparent drawback to this defense system is that it is not complete and levels of AGEs increase steadily with age. One reason is that kidney function tends to decline with advancing age. Another is that macrophages, like certain other components of the immune system, become less active.



Macrophages, such as the one shown here, recognize foreign bodies such as bacteria, or altered molecules such as crosslinked proteins, and remove them from circulation.

Why this happens is not known, but immunologists are beginning to learn more about how the immune system affects, and is affected by aging (**See The Immune System, page 31**). And in the meantime, diabetes researchers are investigating drugs that could supplement the body's natural defenses by blocking AGEs formation.

Crosslinking interests gerontologists for several reasons. It is associated with disorders that are common among older people, such as diabetes and heart disease; it progresses with age; and AGEs are potential targets for drugs. In addition,

cross-linking may play a role in damage to DNA, which is another important focus for research on aging.

DNA Repair and Synthesis

In the normal wear and tear of cellular life, DNA undergoes continual damage. Attacked by oxygen radicals, ultraviolet light, and other toxic agents, it suffers damage in the form of deletions, or deleted sections, and mutations, or changes in the sequence of DNA bases that make up the genetic code. In addition, sometimes the DNA replication machinery makes an error.

Biologists theorize that this DNA damage, which

gradually accumulates, leads to malfunctioning genes, proteins, cells, and, as the years go by, deteriorating tissues and organs.

Not surprisingly, numerous enzyme systems in the cell have evolved to detect and repair damaged DNA. For repair, transcription, and replication to occur, the double-helical structure that makes up DNA must be partially unwound. Enzymes called helicases do the unwinding. Investigators have found that people who have Werner's syndrome (WS), a rare disease with several features of premature aging, have a defect in one of their helicases. George Martin, M.D., of the University of Washington and other investigators are exploring the mechanisms involved in DNA repair in WS and

Werner's Syndrome



WS patient age 14



WS patient age 48

Genetic defects are believed to be responsible for Werner's syndrome (WS), a disease in which people at a young chronological age develop some characteristics associated with aging, such as gray hair, wrinkled skin, diabetes, heart disease, and cancer. Because of the apparent acceleration of aging in WS, gerontologists are delving into its causes, hoping to shed light on the disease and the degenerative processes that occur with normal aging.

"The remarkable increase in our life span during the 20th Century has been due, in large part to the contributions of basic biomedical research...In the 21st Century, the advances in these basic sciences should lead to effective treatments of diseases of our aging population."

—Julius Axelrod, Ph.D.
1970 Nobel Laureate in Medicine

similar disorders, collectively known as progeroid syndromes. This research could help explain why DNA repair becomes less efficient during normal human aging.

The repair process interests gerontologists for many reasons. It is known that an animal's ability to repair certain types of DNA damage is directly related to the lifespan of its species. Humans repair DNA, for example, more quickly and efficiently than mice or other animals with shorter lifespans. This suggests that DNA damage and repair are in some way part of the aging puzzle.

In addition, researchers have found defects in DNA repair in people with a genetic or familial suscepti-

bility to cancer. If DNA repair processes decline with age while damage accumulates as scientists hypothesize, it could help explain why cancer is more common among older people.

Gerontologists who study DNA damage and repair have begun to uncover numerous complexities. Even within a single organism, repair rates can vary among cells, with the most efficient repair going on in germ (sperm and egg) cells. Moreover, certain genes are repaired more quickly than others, including those that regulate cell proliferation.

Especially intriguing is repair to a kind of DNA that resides not in the cell's nucleus but in its mitochondria. These small organelles are the principal sites of metabolism and energy

production, and cells have hundreds of them. Investigators suspect mitochondrial DNA is injured at a much greater rate than nuclear DNA, possibly because the mitochondria produce a stream of damaging oxygen radicals during metabolism. Adding to its vulnerability, mitochondrial DNA is unprotected by the protein coat that helps shield DNA in the nucleus from damage.

Research has shown that mitochondrial DNA damage increases exponentially with age, and as a result, energy production in cells diminishes over time. These changes may cause declines in physiological performance, and may play a role in the development

DNA, the double helix cellular molecule that contains thousands of genes necessary for life, is constantly being damaged and repaired. Gerontologists suspect DNA repair mechanisms become less efficient with age. Accumulating DNA damage, including breaks in its structure or changes in its nucleotide sequences, can lead to some of the physical changes we associate with aging.

HSP-70

of age-related diseases. Investigators are examining how much mitochondrial DNA damage occurs in specific parts of the body such as the brain, what causes the damage, and whether it can be prevented.

Heat Shock Proteins

In the early 1960s, investigators noticed fruit flies did something unusual. When these insects were exposed to a burst of heat, they produced proteins that helped their cells survive the temperature change. Intrigued, researchers looked for these proteins in other animals, and found them in virtually every living thing including

plants, bacteria, worms, mice, and yes, humans. Today, the role of these substances, known as heat shock proteins, in the aging process is under scrutiny.

Despite their name, heat shock proteins (HSPs) are produced when cells are exposed to various stresses, not only heat. Their expression can be triggered by exposure to toxic substances such as heavy metals and chemicals and even by behavioral and psychological stress.

What attracts aging researchers to HSPs is the finding that the levels at which they are produced depend on age. Old animals placed under stress—short-term, physical restraint, for example—have lower levels

of a heat shock protein designated HSP-70 than young animals under similar stress. Moreover, in laboratory cultures of cells, researchers have found a striking decline in HSP-70 production as cells approach senescence.

Exactly what role HSPs play in the aging process is not yet clear. They are known to help cells dismantle and dispose of damaged proteins. They also facilitate the making and transport of new proteins. But what proteins are involved and how they relate to aging is still the subject of speculation and study.

While at the NIA, Nikki Holbrook, Ph.D., and other researchers investigated the

action of HSP-70 in specific sites, such as the adrenal cortex (the outer layer of the adrenal gland). In this gland as well as in blood vessels and possibly other sites, the expression of HSP-70 appears closely related to hormones released in response to stress, such as the glucocorticoids and catecholamines. Eventually, answers to the puzzle of HSPs may throw light on some parts of the neuro-endocrine system, whose hormones and growth factors might have an important influence on the aging process.

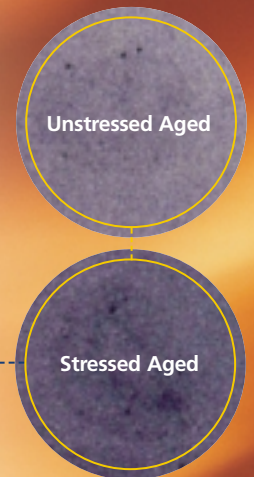
In certain circumstances, plants and animals produce heat shock proteins (HSPs). These proteins are believed to have an important role in helping cells respond to stress. The dark areas in these illustrations show increased expression of HSPs in short term, physically restrained rats. With age, genetic expression of these proteins diminishes.

Heat Shock Proteins

In the Aorta



In the Adrenal Gland



Plants

Since they were first discovered in fruit flies, heat shock proteins have been found in virtually every living thing.

Bacteria



Worms

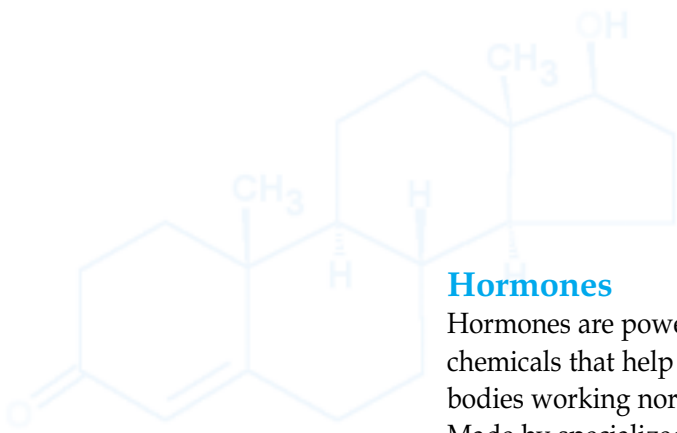


Mice



Humans





Testosterone

Hormones

Hormones are powerful chemicals that help keep our bodies working normally. Made by specialized groups of cells called glands, hormones stimulate, regulate, and control the function of various tissues and organs. They are involved in virtually every biological process including sexual reproduction, growth, metabolism,

and immune function. These glands, including the pituitary, thyroid, adrenal, ovaries, and testes, release various hormones into the body as needed.

As we age, production of certain hormones, such as testosterone and estrogen, tends to decrease. Hormones with less familiar names, like melatonin and dehydroepiandrosterone (DHEA) are also not as abundant in older people as in younger adults. But what influence, if any, these natural hormonal declines have on the aging process is unclear.

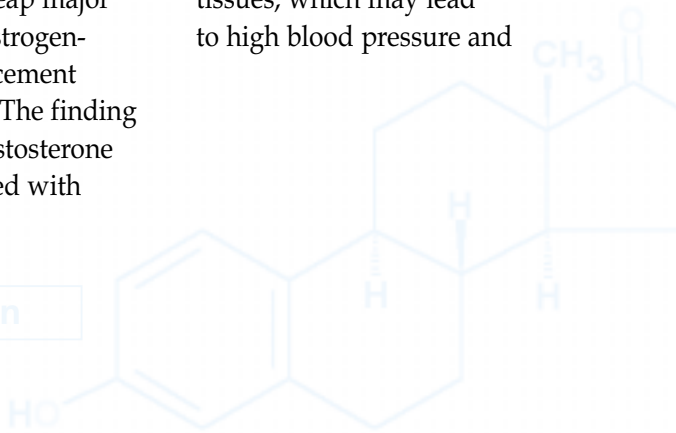
Hormone Replacement

In the late 1980s, at Veterans Administration hospitals in Milwaukee and Chicago, 12 men age 60 and older

began receiving injections three times a week that dramatically reversed some signs of aging. The injections increased their lean body (and presumably muscle) mass, reduced excess fat, and thickened skin. When the injections stopped, these changes reversed and the signs of aging returned. What the men were taking was recombinant human growth hormone (hGH), a synthetic version of the hormone that is produced in the pituitary gland and plays a critical part in normal childhood growth and development. At the same time, evidence was accumulating that postmenopausal women could reap major benefits from estrogen-progestin replacement therapy (HRT). The finding that levels of testosterone in men decreased with

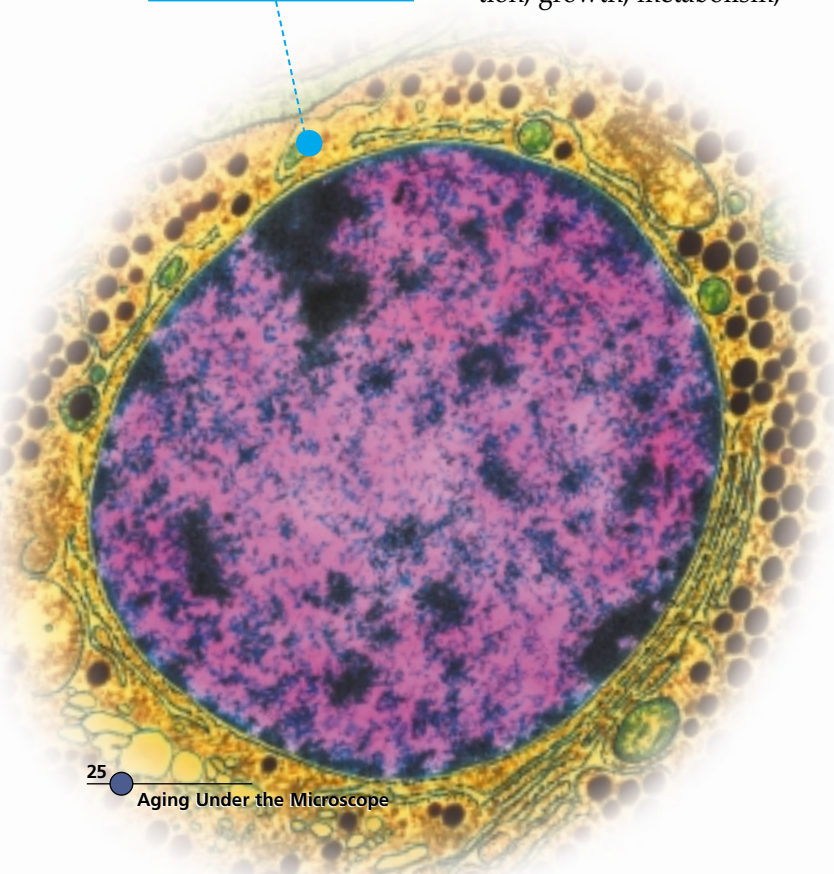
aging raised the question of whether they too might benefit from sex hormone treatment.

As a result of these preliminary findings, the NIA launched a series of research initiatives to clarify what influence hormone replacement therapy might have on the aging process. So far, most of these studies have been inconclusive, but have led many investigators to question whether the risks of hormone replacement may outweigh any benefit. Supplements of hGH, for instance, can promote diabetes, joint pain, carpal tunnel syndrome, and pooling of fluid in the skin and other tissues, which may lead to high blood pressure and



Estrogen

In this colored transmission electron micrograph, growth hormone granules (brown) are visible in the cytoplasm (yellow) of a growth-hormone secreting endocrine cell from the pituitary gland. Visible cell organelles include mitochondria (round, green) and the nucleus (purple, center).





Hormones and Research on Aging

Produced by glands, organs, and tissues, hormones are the body's chemical messengers, flowing through the blood stream and searching out cells fitted with special receptors. Each receptor, like a lock, can be opened by the specific hormone that fits it and also, to a lesser extent, by closely related hormones. Here are some of the hormones and other growth factors of special interest to gerontologists.

ESTROGEN > Although it is primarily associated with women, men also produce small amounts of this sex hormone. Among its many roles, estrogen slows the bone thinning that accompanies aging and may help prevent frailty and disability. In premenopausal women, the ovaries are the main manufacturers of estrogen (**image above**). After menopause, fat tissue is the major source of a weaker form of estrogen than that produced by the ovaries. While many women are helped by estrogen replacement therapy during and after menopause, some are placed at higher risk for certain diseases if they take it. As research yields more information about this carefully studied hormone, it may help clarify who should take estrogen replacement supplements and who should not.

GROWTH HORMONE > This product of the pituitary gland appears to play a role in body composition and muscle and bone strength. It is released through the action of another trophic factor called growth hormone releasing

hormone, which is produced in the brain. It works, in part, by stimulating the production of insulin-like growth factor, which comes mainly from the liver. All three hormones are being studied for their potential to strengthen muscle and bones and prevent frailty among older people. For now, however, there is no convincing evidence that growth hormone supplements will improve the health of those who do not suffer a profound deficiency of this hormone.

MELATONIN > Contrary to some claims, secretion of this hormone, made by the pineal gland, does not necessarily diminish with age. Instead, a number of factors, including light, can affect production of this hormone, which seems to regulate various seasonal changes in the body. Current research does indicate that melatonin in low dosages may help some older individuals with their sleep. However, it is recommended that a physician knowledgeable in sleep medicine be consulted before self-medication. Claims that melatonin can slow or reverse aging are far from proven.

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continued >> Hormones and Research

TESTOSTERONE > In men, testosterone (**image below**) is produced in the testes (women also produce small amounts of this hormone). Production peaks in early adulthood. However, the range of normal testosterone production is vast. So while there are some declines in testosterone production with age, most older men stay well within normal limits. The NIA is investigating the role of testosterone supplementation in delaying or preventing frailty. Preliminary results have been inconclusive, and it remains unclear if supplementation of this hormone can sharpen memory or help men maintain stout muscles, sturdy bones, and robust sexual activity. Investigators are also looking at its side effects, which may include an increased risk of certain cancers, particularly prostate cancer. A small percentage of men with profound deficiencies may be helped by prescription testosterone supplements.

DHEA > Short for dehydroepiandrosterone, DHEA is produced in the adrenal glands. It is a precursor to some other hormones, including testosterone and estrogen. Production peaks in the mid-20s, and gradually declines with age. What this drop means or how it affects the aging process, if at all, is unclear. Investigators are working to find more definite answers about DHEA's effects on aging, muscles, and the immune system. DHEA supplements, even when taken briefly, may cause liver damage and have other detrimental effects on the body.

heart failure. Studies in mice have raised other concerns about the hormone. Investigators have found that mice deficient in growth hormone production live substantially longer than normal mice, while mice overproducing growth hormone live shorter than average lives. This finding suggests that even if hGH replacement therapy is initially beneficial, ultimately it may be harmful, and actually might curtail longevity.

Similarly, there is scant evidence that testosterone supplementation has any positive impact in healthy older men. In fact, some studies suggest supplementation might trigger excessive red blood cell

production in some men. This side effect can increase a man's risk of stroke. Estrogen, perhaps the most well-studied of all hormones, certainly may be helpful for some postmenopausal women. Investigators, however, are finding estrogen replacement therapy might not be a panacea. In fact, long-term use (more than 5 years) of estrogen supplements slightly increases a woman's risk of developing breast cancer. Estrogen supplements also may raise a woman's risk of getting blood clots, which can block blood circulation in arteries.

As research continues, the pros and cons of hormone replacement may become more precisely defined. Other than estrogen, the majority of these supplements




appear to have few clear-cut benefits for healthy individuals, and no proven influence on the aging process.

Growth Factors

Some types of hormones can be referred to as growth or trophic factors. These factors include substances such as insulin-like growth factor (IGF-I), which mediates many of the actions of hGH. Another trophic factor of interest to gerontologists is growth hormone releasing hormone, which stimulates the release of hGH. Growth factors might have an important role in longevity determination. In nematodes, for instance, mutations in at least two genes in the IGF-I pathway result in extended lifespan.

The mechanisms—how hormones and growth factors produce their effects—are still a matter of intense speculation and study. Scientists know that these chemical messengers selectively stimulate cell activities, which in turn affect critical events, such as the size and functioning of skeletal muscle. However, the pathway from hormone to muscle is complex and still unclear.

Consider growth hormone. It begins by stimulating production of IGF-I. Produced primarily in the liver, IGF-I enters and flows through the blood stream, seeking out special IGF-I receptors on the surface of various cells, including muscle cells. Through these receptors it signals the muscle cells to increase in size and number, perhaps

 Friends...drinking lots of good water, no alcohol, staying positive, and lots of singing will keep you alive for a long time."

—Chris Mortensen (1882-1998),
America's oldest man, on his 115th birthday.

by stimulating their genes to produce more of special, muscle-specific proteins. Also involved at some point in this process are one or more of the six known proteins that specifically bind with IGF-I; their regulatory roles are still a mystery.

As if the cellular complexities weren't enough, the action of growth hormone also may be intertwined with a cluster of other factors—exercise, for example, which stimulates a certain amount of hGH secretion on its own, and obesity, which depresses

production of hGH. Even the way fat is distributed in the body may make a difference; lower levels of hGH have been linked to excess abdominal fat but not to lower body fat.

Like many hormones, blood levels of progesterone, a reproductive hormone, (right) diminish with age. But what influence, if any, the natural decline in progesterone and other hormones has on the aging process in middle and late life is unclear.

