Gerontology Research Comes of Age

The tools of molecular biology have begun to make it possible to separate aging from disease—ultimately offering the hope of extending our lives

IN THE PAST 125 YEARS, THE LIFE EXPECTancy of Americans has almost doubled: from 40 to nearly 80 years. This success has raised hopes that modern medicine and science could stave off mortality even further, perhaps creating a race of disease-free centenarians. But an article in this issue of *Science* (see page 634) concludes that the "easy" gains in life expectancy have already been made. Most of those gains have come through a combination of reducing deaths of the young (particularly infants) and mothers in childbirth. Remarkably enough, the authors of the report conclude that, even cures for cancer and heart disease won't be enough to increase life expectancy another 20 years.

So what would increase life expectancy? According to the report's authors, a team from the University of Chicago and the Argonne National Laboratory, the only likely answer is basic research at the molecular level aimed at preventing the degenerative diseases of old age—or actually postponing the aging process itself. And that's just what a growing number of gerontological researchers are working on. Using the new tools of molecular biology, they are teasing out the many factors that cause cells to "senesce." In this process they are beginning—for the first time—to separate the pathologies of age from the normal process of aging. The researchers doing this work stress that it remains very basic and far from application. But they have hope that it will one day be used to lengthen—and improve—the end of the human life-span.

> ***"If you thought cancer was complex, look at aging."***
>
> —Edward Schneider

One sign of the maturity of the field is that, after years of being considered a lesser, applied science, gerontology is finally getting both more respect and more funds. "If you're a researcher, you can't go into a better field. We're at the point now that the lay public is recognizing the importance of research in aging, and the funding from the government sector and the private sector is increasing," says Edward L. Schneider, dean of the Ethel Percy Andrus Gerontology Center at the University of Southern California.

Although the National Institute on Aging's tentative 1991 budget of $325 million ranks eighth out of the 13 health institutes (with about 45% of that budget going to the study of Alzheimer's disease), the appropriations for the institute increased 36% this year, more than twice as much as any other institute at NIH. In addition, pharmaceutical companies are now spending almost half of their R&D dollars—$3.6 billion—on aging, according to the Phar-

A New Bestiary for Aging Research

"It's like trying to understand human psychology by interviewing two brothers," says Harvard biologist Steven Austad. Austad is criticizing gerontology's traditional reliance on laboratory mice and rats to study the aging process.

Austad isn't alone. In one of the most interesting trends in aging research, he and others are turning their attention away from the "two brothers" to a curious menagerie that includes spiders, lizards, opossums, turtles, bats, Japanese quail—even platyfish.

The result, many researchers believe, will be a fundamental advance in gerontology. "I predict. . . a revolutionary change in how lab-based scientists think about senescence and their choice of models for senescence studies," says Caleb Finch, a neurophysiologist at the Ethel Percy Andrus Gerontology Center at Los Angeles' University of Southern California.

Such changes are already in the wind. One of the most promising new models has long been favored by developmental biologists: the fruit fly. *Drosophila* breeds rapidly and scientists already know a great deal about its genetic makeup. Hence "it's the perfect animal for aging studies," says Michael Rose, an evolutionary biologist at the University of California at Irvine, who has relied on the fruit fly to test aspects of the evolutionary theory of aging.

That theory predicts a correlation between the age at which an animal reaches maturity and the onset of aging. *Drosophila*, which breeds almost as soon as it matures (at about 5 days), dies about 25 days later. But by selectively breeding fruit flies that matured late in life, Rose has succeeded in expanding their life-spans by 100%—and he predicts achieving a 200% increase. "It's not just an increase in life-span," he notes, "but an actual postponement of aging. Their ability to reproduce and to fly are enhanced at older ages. It would be like being 120 years old and still capable of playing a good game of tennis."

In Michael Hadfield's University of Hawaii laboratory, the sea slug (*Phestilla sibogae*), which inhabits a specific Hawaiian coral, has benefited in a similar manner by delaying the onset of reproduction. After hatching, the small creature normally spends about 3 days as a free-swimming larva, then settles onto the coral and metamorphoses into an adult slug. Its next 60-odd days are devoted to eating the coral and reproducing, and when its fertility is spent, the sea slug dies. Hadfield's slugs, however, gained an additional 30 days by living in a state of almost suspended animation.

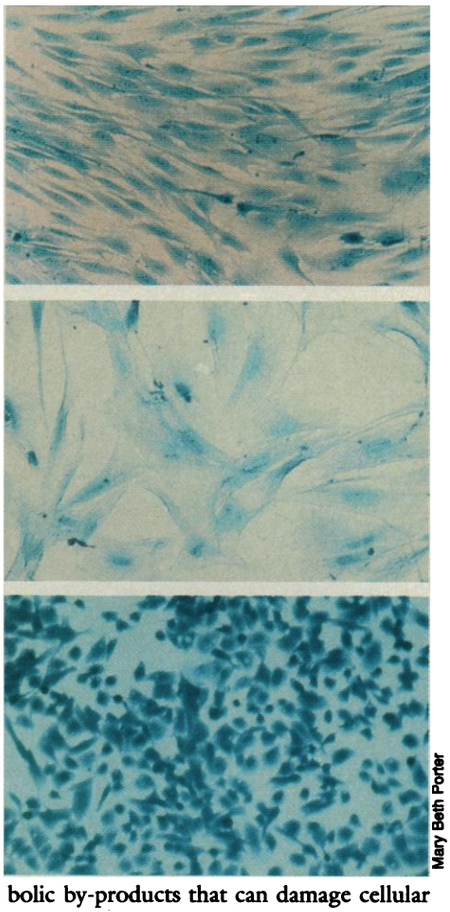
"We don't give the larvae a place to land, so they keep swimming, and they can stay like that for a month or more," says Hadfield. When Hadfield does give them a coral home, the slugs continue their lives as if nothing unusual had happened. "They still get their full reproductive span of time," Hadfield says, "which basically means that for an entire month, their biological clocks stopped ticking."

maceutical Manufacturer's Association.

Much of this new excitement in the field of gerontological research has been generated by the possibility of distinguishing between disease and ordinary aging. Researchers know that disease isn't a necessary part of aging, but the two are very difficult to disentangle, because most people do get various diseases as they age. As a result, "if you ask what's normal aging, we still can't tell you," says Huber Warner, chief of the molecular and cell biology branch at the NIA.

But the hope that normal aging will soon be described is what is driving many gerontology researchers. Although many previous theories were based on factors specific to old age—such as a single "aging gene" that is turned on late in life—little evidence has been gathered to support such theories, and momentum in the field has been swinging to theories saying aging is simply the result of cells becoming less efficient at self-repair as they age. "If aging isn't genetically programmed, then it must be the result of normal by-products of metabolism and development that are necessary to life," says Richard G. Cutler, a research chemist at the NIA's Gerontology Research Center in Baltimore. "Aging is passive: What causes aging is being alive."

Yet if aging is merely the accumulation of cellular wear and tear, the buildup of lapses in housekeeping, then the process will undoubtedly turn out to have many causes. And understanding these multiple factors is the great current challenge of gerontological research, which brings together immunologists, geneticists, endocrinologists, chemists, biologists, and physicians who specialize in degenerative diseases. Says Schneider: "Aging research is not for the weak at heart. If you thought cancer was complex, look at aging."



Mary Beth Porter

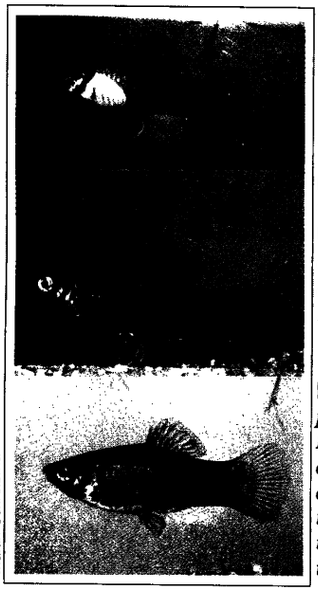
Is aging only skin deep? *Normal (top), senescent (middle), and immortalized (bottom) fibroblasts show distinctly different appearances. The fibroblast is a skin cell much used in culture in research on aging.*

But there are common threads that tie the disparate, interdisciplinary research together. One of those threads is the fact that normal cell lines have a fixed life-span. Human fetal fibroblasts, for instance, will divide some 50 times in culture before stopping. No one knows what the mitotic off-switch might be, and much of the current work in gerontology is aimed at identifying the switch and the changes that go on in cell cultures as they reach the end of their span.

One theory that has become a contender in the last decade might be called the "garbage can" hypothesis. As a cell line ages, this theory goes, it turns into a sort of multigenerational wastebasket: somehow later generations of the line accumulate metabolic by-products that can damage cellular macromolecules, including nucleic acids and proteins. Furthermore, unlike cells earlier in the lineage, those later in the cell line seem to be less efficient at repairing damage.

Hadfield notes that unlike calorie restriction studies, which have been used to increase the life-spans of creatures as disparate as spiders and mice, his research never disturbs the sea slug's physiology. "We're just varying the moment when they find coral and reproduction can begin."

The tiny platyfish (*Xiphophorus maculatus*) can also be "reprogrammed" for a longer life—although in this case, early rather than late maturity is the key. "We've identified a particular puberty gene," says Martin Schreibman, a comparative neuroendocrinologist at New York's Brooklyn College, "and can use that to generate fish that mature at different ages. Those that mature early, at about 18 weeks, live about 10 months longer than the others."



Martin Schreibman

Fish story. *Three male platyfish siblings bred by Martin Schreibman each carry a different allele for maturation. The earlier the fish mature, the smaller they are when they stop growing—and the longer they live.*

Schreibman says he selected platyfish as a model because, from the point of view of the gerontologist at least, they show a "striking similarity" to humans. What he means is that, unlike most fish, platyfish are internal fertilizers who carry their young until they are free-swimming. He has been able to double the fish's life by raising them in isolation. While admitting that this ploy probably is "not that much fun," Schreibman believes it points to a neuroendocrinal relationship between the fish's lifespan and its environment.

For the Japanese quail (*Coturnix coturnix japonicus*), reproductive failure—one of the key indicators of senescence—typically happens when the bird is 18 months old. But even before this, as early as 1 year, male quails reveal another aging clue: they stop their courtship displays.

"You can tell exactly when old age sets in by watching the changes in mating behavior," says Mary Ann Ottinger, a behavioral endocrinologist at the University of Maryland. "Subsequently—and contrary to what we predicted—their hormone levels decline and they get increasing numbers of gonadal tumors. So we are seeing a behavioral change in the absence of a hormonal change, which means that something else in the system is breaking down."

Male quails that are injected with hormones will once again act like romantic youngsters—resuming their strutting and courting calls and beginning to mate. "The question is, what are you turning on?" asks Ottinger. "What remains plastic enough to be

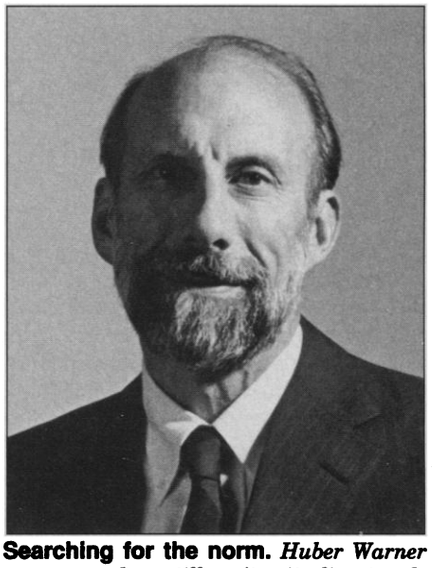
Proponents of the garbage can approach are looking at intracellular debris to understand how that waste might interact with the cell line's genome. One significant form of garbage may be the molecules called oxygen-free radicals. In cell metabolism, oxygen forms superoxides, a highly reactive oxygen species with an extra electron that allows it to bond readily with other chemical groups. The havoc-wreaking radicals invade the cell's membrane and attack its fats and proteins, forming hydrogen peroxides and lipid hydroperoxidases that also are destructive. According to a theory proposed by Denham Harman of the University of Nebraska in the 1950s, they can also damage the DNA.

It has been shown that when a cell line is young, housekeeping enzymes known as antioxidants disarm the free radicals. Cutler and his colleagues at NIA are pursuing the possibility that these scavengers may be a clue to comparative longevity among species. They have already shown that longer lived species have more antioxidants than their shorter lived brethren. Cutler's current theory is that in aging cells, antioxidants may be neutralized or overwhelmed by an increasing number of free radicals, which may prevent the cell from dividing and differentiating. Alternatively, he says, later generations of cells may be less efficient at producing the beneficial enzymes.

Another destructive agent may be glucose, which attaches itself to proteins in a process known as non-enzymatic glycosylation. The protein-sugar complex sets in motion a chain of chemical reactions that cross-link adjacent proteins, sticking them together in a yellowish-brown mass known as advanced glycosylation end products (AGEs). As people age, AGEs gum up the tissues, making them stiffer and less elastic and causing the connective tissue to get leathery, according to Anthony Cerami, the Rockefeller University biochemist who coined the term AGEs. Like free radicals, AGEs may interact with DNA, causing mutations that interfere with the cell's ability to repair, replicate, and transcribe its DNA.

In fact, it's difficult for researchers to figure out which comes first: a glut of DNA-damaging glucose and free radicals or a genetic defect that interrupts the housekeeping mechanisms that normally keep corrosive agents in check. As researchers attempt to puzzle out the dynamics of this system, they have discovered a growing number of genes that are implicated in the aging process. Some genes seem to have a very direct role in helping organisms to live longer. These are what Cutler calls longevity determinant genes. He's studied 20 different species, from mice to human beings, and in every instance there is evidence for genes that determine the overall metabolic rate. Since the faster a cell metabolizes, the more free radicals are produced, these genes may have a powerful effect on the buildup of the cellular "garbage."

Support for the idea of "aging" genes has come from work in a whole bestiary of new animal models that augment the traditional reliance on mice and rats in gerontology research (see box on page 622). Each of those animals has something to contribute to the study of aging, and they may ultimately transform the field of gerontology.



Searching for the norm. ***Huber Warner says researchers still can't quite disentangle normal aging processes from disease.***

stimulated like that again? Whatever it is seems to have been lost in mammals."

All these results, intriguing as they are, come from the laboratory bench. Outside the laboratory, other scientists are seeking to understand how—and in some cases, whether—animals change physiologically at the end of their life-spans in the wild. "There is really very little information about how animals age in nature," says Raymond Huey, a herpetologist from the University of Washington in Seattle. "Nor is it clear if athletic performance in animals has any value in nature. Do high-performance animals get the equivalent of gold medals by surviving longer or getting more mates?"

Huey turned to a population of canyon lizards (*Sceloporus merriami*) in southern Texas whose ages were already known. He ran them through a series of treadmill and burst speed tests. While a little slow at the gate, older lizards showed more stamina than those in their youth, but this endurance did not promise them a longer life. "They do age, and as they age, their mortality rates increase," says Huey.

Yet other animals, notably freshwater turtles and certain seabirds, display no evidence of senescence even though they have life-spans similar to those of humans. "A turtle that is 50 years old will lay as many eggs as one that is 15," notes J. Whitfield Gibbons, a herpetologist at the Savannah River Ecology Laboratory in South Carolina, who is tracking several populations of freshwater turtles. "There is no apparent change in the health of the older individual; no greater susceptibility to disease. And when it dies, it will be because something has killed it, not because of old age. I can't guarantee that senescence doesn't occur, but it's not apparent."

Harvard's Steven Austad suspects that the turtles' environment and protective shell play some role in their long life, just as an island environment apparently extends the life of typically short-lived opossums. On Georgia's mainland, Austad observes, opossums live only 1.3 years. But on an offshore island, opossums live substantially longer—in some cases up to 3 years—perhaps because there are no predators or cars.

"The evolutionary theory of aging holds that the rate at which animals age is related to the rate at which they die either from predators or in accidents," says Austad. Thus, the mainland opossums, facing death every time they cross an interstate, find it much more to their advantage to reach maturity quickly and reproduce—but in so doing, they also age rapidly.

Overall, the comparative method offers a way for researchers to understand aging in general, as an evolutionary force that plays a role in the life history of all animals. "It may be that none of the lessons we learn from these animals are directly applicable to ourselves," says Huey. "But simply by bringing people into aging research who approach it from a different perspective will change the way gerontologists do research."

■ VIRGINIA MORELL

Virginia Morell is a free-lance writer based in Ashland, Oregon.

One of the great virtues of some of these new models is that they can be easily manipulated genetically. In human beings, of course, researchers cannot do comparable manipulations. So instead they have turned to work on human cells in culture.

Most of that work has been done using fibroblasts, which, since the 1950s, have been considered a good in vitro model for aging. At the NIA, David Danner and his colleagues took advantage of this model to test the theory that a protein called prohibitin inhibits the division of cells. In an elegant experiment, the researchers micro-injected prohibitin messenger RNA into actively growing cells, which then stopped proliferating. The conclusion was that prohibitin inhibits cell division and may play a part in cell senescence.

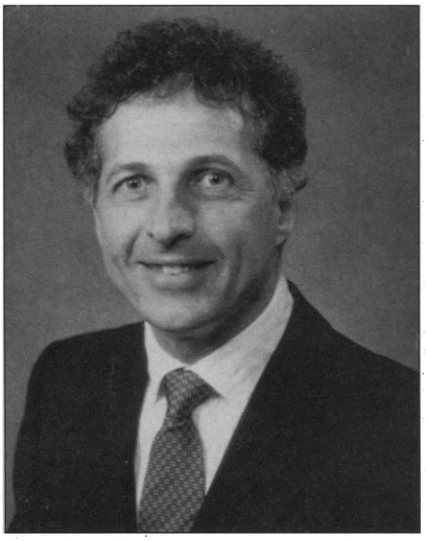
But if the inhibition of cellular proliferation is a key factor in aging, then it would seem likely that genes with a role in cancer—which is a form of uncontrolled proliferation—also have something to do with aging. And indeed, recent work crosses the boundary between cancer research and gerontology. For example, the oncogene c-*fos* must be activated to permit cell proliferation, and Judith Campisi, a cell biologist at UC Berkeley, has shown that c-*fos* is not expressed in senescent cells.

The connections between cancer and aging are deepened by findings related to the retinoblastoma (RB) gene, a tumor suppressor that also controls expression of c-*fos*. Experiments by Gretchen Stein at the University of Colorado at Boulder show that growth factors inactivate the RB protein in young cells, but fail to do so in aging cells—suggesting that the failure to inactivate the RB protein is an important step on the road to stopping the cells from dividing, which may be an aspect of senescence.

Stein's work set the stage for experiments by Samuel Goldstein of the Veterans Administration Hospital in Little Rock, Arkansas, and the University of Arkansas, who has searched for mechanisms that regulate senescent cells and RB gene expression. In that work Goldstein has been using cells from humans with Werner's syndrome, a rare disorder whose victims show signs of advanced aging in their 20s. Their cells seem to provide a good model for aging because they divide only 10 to 20 times, rather than the 50 divisions seen in normal human cell lines.

Goldstein has screened the Werner's cells and finds they are drowning in excess proteins such as collagen and fibronectin (a protein that anchors cells in the extracellular matrix). That work is intriguingly consistent with earlier observations by James R. Smith and Olivia Pereira-Smith at Baylor College of Medicine, who found that fibronectin exists in large amounts in senescent cells.

The genes that encode collagen and fibronectin may have a role in the aging process, but if they do, they are almost certainly only a small part of a much larger genetic cascade. Many of the genes in that cascade are likely to be regulatory genes, and several groups are hot on the trail of the regulators.



Maturing field. *According to Edward Schneider, gerontology research is finally getting some respect—and more funding.*

"I'd like to believe that genes clearly are regulated differently in old cells and young cells," says Goldstein. At least two different types of genetic events are likely to be involved: the turning on of genes that block cell division and the turning off of genes that normally stimulate cell growth. Goldstein calls this combined genetic turning off and turning on a kind of cellular "yin and yang."

Although these results, based on fibroblasts in culture, are tantalizing, not all researchers are convinced that what happens in fibroblasts in culture truly reflects what is going on in the aging human being. Richard A. Miller of the University of Michigan thinks the studies with fibroblasts are interesting, but "until they can demonstrate that long-term changes in fibroblasts are also happening in the aging skin of older people, I don't think they're telling us anything about aging."

Miller argues that a more reliable model for human aging is immune cells, because more is known about the workings of the immune system than about what goes on in other types of somatic cells. Most researchers are convinced that the immune system slackens with age, and they have systematically described how it becomes less diligent in its surveillance of foreign cells. A major factor is the impairment of the T and B lymphocytes, which become slower in responding to stimuli. That may be because aging immune system cells produce far less interleukin-2, a key T cell growth factor—a fact that was demonstrated by Marc Weksler's lab at Cornell University and Miller's at Sloan Kettering (among other groups) in the 1980s.

More recent work in the lab of Bill Weigle at the Research Institute of Scripps Clinic in La Jolla has shown that the immune systems of aging mice can be restored in part when they are given huge doses of IL-2. This suggests an approach for boosting the aging immune system in human beings, and "IL-2 cocktails" are currently under development at several pharmaceutical companies. "The immune system is sufficiently well understood at this point that it's an obvious place for intervention," says Miller.

In fact, there are many obvious places to intervene in the chain of events that causes aging, because that process occurs on so many levels—from molecules to organs. And that's why the pharmaceutical companies are plunging billions into research in this area—although the details of their projects are mostly closely guarded secrets.

Perhaps the ultimate hope is a form of gene therapy that might interrupt the cascade of genetic events that leads to aging. "It's obvious that an intimate understanding of the events that lead to senescence would tell you which genes are important and how they are regulated, and how you can begin to change the gene regulation to make the cells live longer or restore their normal function," says Goldstein.

That is, of course, little more than a faint hope for a distant future. Yet, if one takes the very long view, it begins to seem like a more exciting possibility than such problems as, say, cancer. "We'll have cures for many cancers soon...and for other diseases," says Schneider in his bluest of blue-sky modes. "I think the real research opportunity, the real excitement, the real puzzle to be solved, will be aging."

■ ANN GIBBONS

ADDITIONAL READING

J. Campisi *et al.*, "Repression of c-*fos* transcription and an altered genetic program in senescent human fibroblasts," *Science* **247**, 205–209 (1990).

A. Cerami *et al.*, "Glucose and aging," *Sci. Amer.* **256**, 90 (May 1987).

S. Goldstein, "Replicative senescence: The human fibroblast comes of age," *Science* **249**, 1129 (1990).

R. A. Miller, "Minireview: The cell biology of aging: immunological models," *J. Gerontol.* **44**, B4-8 (1989).

J. R. Smith, "Minireview: DNA synthesis inhibitors in cellular senescence," *ibid.*, **45**, B32-35 (1990).

G. Stein *et al.* "Failure to phosphorylate the retinoblastoma gene product in senescent human fibroblasts," *Science* **249**, 666 (1990).