Ribbons of elastin, a protein that helps keep skin supple, magnified 1,000 times.

Wrestling With Wrinkles

Scientists are closing in on the secrets of skin aging

By RICK WEISS

Beauty will be ravaged by age, and the face that charmed will be ploughed by wrinkles. The time will come when it will vex you to look at a mirror, and grief will prove a second cause of wrinkles. — Ovid

Ancient Rome's poet laureate of love never heard of topical tretinoin, or Retin-A, the antiwrinkle cream that recently has gained medical and popular celebrity. But his words document skin wrinkling as an age-old concern, and help explain the marketplace mania that followed publication of research earlier this year showing tretinoin's reversal of "dermatoheliosis," or sun-induced skin aging.

Sales of the drug have "skyrocketed" since publication of that research in the Jan. 22/29 JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION (JAMA), according to its manufacturer, Ortho Pharmaceutical in Raritan, N.J. The article featured striking, full-color "before and after" pictures of tretinoin-treated skin that showed a marked reduction in the number of wrinkles and an obvious fading of "age spots" and sunlight-induced freckles.

Scientists still don't understand the mechanism by which tretinoin exerts its effects. Indeed, the phenomenon of aging itself remains one of the great mysteries of biological science. But inspired by tretinoin's apparent ability to reverse at least some of the obvious signs of skin aging, and equipped with a new generation of mechanical and molecular biological tools, dermatologists at last are gaining insight into the enigmatic relationship between biology and time. And while skin aging is of obvious interest to anyone concerned about personal appearance, its study appeals to more than simple vanity.

"Many of the processes associated with aging are the same both in the skin and in other tissues," says Jouni J. Uitto, chairman of the department of dermatology at Jefferson Medical College in Philadelphia. Aging research has often focused on the skin, if for no other reason than "the skin is very accessible."

Moreover, says Richard G. Cutler, a research chemist with the National Institute on Aging, the skin performs a broad range of biological functions. So an understanding of how it ages—and of how to slow that process — may add to our understanding of age-related degeneration in a variety of other organ systems. In many ways, he says, skin is an ideal model of biological deterioration.

"A skin cell, before it falls on the floor, has a predestination with death," Cutler says flatly. But senescence is not simply a matter of running out of cells or enzymes, he adds. "Aging is more subtle than that."

Subtlety, of course, is in the eye of the beholder, and the aging process may seem anything but subtle to the owner of a wrinkled integument. To a dermatologist peering into a microscope, the situation looks even worse.

Aged skin is thinner than younger skin, and is characterized by an increase in cellular disorganization, says Lynne T. Smith of the University of Washington in Seattle. Where healthy basal cells once stood lined up in neat columns within the epidermis, or outer layer of skin, scientists find them in disarray—evidence of a breakdown in the normal process of cellular proliferation and organization.

With age, collagen fibers — important in maintaining skin integrity — decrease in number, organization and density. Smooth, ribbon-like fibers of elastin, responsible for skin's ability to "snap back" to shape after being stretched, get coarser, denser and less resilient, especially where the skin has been exposed to sunlight.

Tiny blood vessels in the dermis, the deeper layer of skin, become thick-walled yet leakier, and there is a general loss of hair, nerve cells, sweat ducts and sebaceous glands that produce fatty secretions called sebum.

In short, says Albert Kligman, a dermatologist at the University of Pennsylvania in Philadelphia, "the whole busi-

ness of [skin] aging is detrimental. It's thumbs down." Nonetheless, he adds, pointing to a picture of a sun-wrinkled woman whose face resembles a severely dried apple, "no person should ever have to look like this."

The implication is that while some aspects of aging may be unavoidable, many age-associated biological changes are not. In the case of skin and probably many other parts of the body, say Kligman and others, such changes — especially those triggered by sunlight or certain oxygen-dependent reactions — can be prevented and even reversed.

Skin aging can be thought of as a combination of two processes: intrinsic, chronological aging, which appears to be a genetically programmed senescence; and extrinsic, accumulated environmental damage such as photoaging — the result of a series of chemical reactions triggered by exposure to sunlight.

Scientists remain uncertain whether anything can be done to alter the course of intrinsic aging. Recent research using molecular biological techniques on cultured cells shows that individual cells — unfamiliar as they may be with the concept of time — seem to "know" how old they are, and age in predictable ways.

"Cells don't actually measure time," notes Vincent Cristofalo, director of the Center for the Study of Aging at the University of Pennsylvania in Philadelphia. "Aging isn't measured by the passage of time, but rather by some sort of sequential molecular events that the cell can understand, but which we can only keep track of by measuring the rotation of the Earth on its axis or its movement around the sun."

New research indicates that after a skin cell has lived out the lifetime allotted by its genetic code, certain membrane receptors in the cell become insensitive to growth factors that normally trigger DNA replication and cell division.

"Proliferation is the most basic and obvious ability that cells lose with age," Cristofalo said at a recent symposium on the biology of skin aging, convened at Jefferson Medical College. "With age, there's a lengthening of generation time until an arrested state."

In contrast to this seemingly inevitable decline, photoaging — which can lead to a cell's premature demise — is preventable and apparently largely reversible, scientists are finding. This is good news for those who want to have young-looking skin, since photodamage, rather than intrinsic aging, causes most of the visible signs of aging, such as pigmentation changes and wrinkles.

Photoaging results from a variety of interactions between sunlight and skin, in which light energy from the sun takes both a direct and indirect toll inside keratinocytes, fibroblasts and other skin cells. In a typical photoaging reaction, any one of a number of biological molecules inside a cell may become electronically excited after exposure to solar energy, transforming it into what's known as a high-energy "singlet." A singlet may then release some of its excess energy through a series of intracellular processes, which can result in a breakup of nearby chemical bonds. In this way, cell membranes can be directly damaged within a few minutes of exposure to sunlight, especially if that light is rich in the shorter-wavelength ultraviolet (UV) range — the so-called UVB, as opposed to UVA or visible light.

Amino acids (the building blocks of proteins and enzymes) and nucleic acids (components of DNA and RNA) are the primary light-absorbing intracellular molecules damaged by ultraviolet light. In addition, sunlight can trigger a reorganization of chemical bonds — a process called cross-linking — within collagen and elastin fibers, and between certain proteins and DNA, leaving them structurally and functionally crippled. Particularly susceptible to photodamage are proteins rich in the amino acid cysteine or otherwise reliant on weak, hydrogen bonds for their three-dimensional structures.

Sunlight also can have indirect damaging effects, in which energy is absorbed by oxygen to form so-called oxygen free radicals such as superoxide anions. These highly charged compounds are not solely the products of sunlight-depend-

Wrinkle Measurement: Art or Science?

Scientists traditionally are skeptical about drugs or cosmetics that purport to reverse the aging process. Beyond their intuitive knowledge that time marches only forward, researchers are hampered by a lack of objective tools for measuring the characteristics of youthful-looking skin such as color, elasticity and degree of wrinkling.

"Our clinical research is a little unusual in that we have yet to develop protocols," says E. George Thorne, director of clinical research at Ortho Pharmaceutical, tretinoin's manufacturer. "We haven't developed assessment techniques. We think we have drugs now that are effective, [but] the real problem we're facing is how to prove it."

In the landmark, double-blind trials of tretinoin published in JAMA earlier this year, dermatologists relied almost exclusively on simple and somewhat inconsistent "before and after" photographic comparisons. Since then, the researchers have developed more sophisticated photographic systems for making standardized comparisons of skin appearance over time.

But in an attempt to stay on more solid scientific ground, researchers are turning to ever more complex apparatuses to gauge the clinical — as opposed to chronological — age of living skin. So-called profilometric devices, for example, resemble computerized, stereo-system tone arms. They record the tiny, cell-sized hills and dales along a styrene surface that has been molded from a silica imprint of a person's skin. Thus researchers can store a computer record of the microterrain of a patient's wrinkled skin for comparison with subsequent samples.

Other devices use technology developed for satellite analysis of the Martian landscape. They scan the skin optically and analyze shadow patterns to measure the depth and density of wrinkles.

A number of seemingly tortuous contraptions measure various mechanical properties of skin. Among them are indentometers (for measuring the compressibility of skin), extensometers (for measuring the force required to extend skin a certain distance) and turgometers — devices that essentially emulate the physician's pinch.

In addition, dermatologists use a variety of methods that twist the skin to measure elasticity, and they are experimenting with ultrasound to measure the thickness of different layers of skin.

Skin Study Center

The L'Oréal Twistometre, a painless, skin-gripping, constant-torque motor, is used to measure how far the skin will twist and how quickly it will snap back into place.

Hardware alone is not the answer, Thorne adds. Terminology must be better defined as well. "If you're going to do a multicenter study, every investigator has to know what pinkness is. Roughness, too, is something you have to define . . . and you also have to define what 'much improved, improved, slightly improved and no change or worse' actually mean." — *R. Weiss*

ent reactions; they also accumulate as normal by-products of oxygen metabolism. Neither are they exclusively harmful. "Many good, normal reactions in the body are mediated by oxygen free radicals," says Cutler.

But whether produced by sunlight-mediated reactions in the skin or by simple oxidative metabolism in the body, these "active oxygen species" appear to be primary culprits in the aging process. It's likely, Cutler says, the relatively long lifespan characteristic of humans may be due to higher levels of antioxidative protective factors and, to a lesser extent, our ability to repair damaged DNA quickly.

Perhaps not surprisingly, tretinoin belongs to a class of vitamin-A-related compounds, known as retinoids, renowned for their ability to quench oxygen free radicals. Dermatologists are increasingly convinced that a better understanding of protective factors such as tretinoin and other antioxidants such as beta carotenes may be the key to slowing not only photoaging, but metabolic oxygen-dependent skin aging as well.

"Screens out harmful ultraviolet rays, conditions skin, repels insects, won't wash off while swimming, will not stain most fabrics. Warning: Contact with eyes, ears, nose, or mouth may be fatal."

Wrinkles and "age spots" are primarily the result of sunlight-induced aging of the skin. Despite recent improvements in their ability to shrink wrinkles and otherwise reverse some of the sun's skin-damaging effects, dermatologists continue to recommend sunscreens as the first and best line of defense against photodamage. Indeed, they assert, those who mistakenly fear sunscreens more than they fear photodamage are doomed to wrinkled – and perhaps cancerous – skin.

Its recent rise to fame notwithstanding, tretinoin is not new to seekers of smoother skin. In 1971 it gained U.S. Food and Drug Administration approval as a treatment for severe acne, and for years patients reported to their dermatologists that the ointment seemed to erase wrinkles while clearing up their acne. But only in the past year have researchers reported results of controlled human studies showing definite improvement in the appearance of photodamaged skin.

Earlier experiments on sunburnt, hairless mice showed that even without treatment, an appreciable amount of repair can occur following photodamage, including deposition of new collagen fibers. "Once you eliminate the photo-insult, the skin can reconstitute itself to a remarkable degree," says Lorraine Kligman, a research assistant professor at the University of Pennsylvania in Philadelphia and Albert Kligman's wife. "So we began to think that if the skin can repair itself, can we help it along pharmacologically?"

Experiments using different tretinoin-like compounds on the mice confirmed the possibility of speeding the recovery of sun-damaged skin. Following a cutback of sun exposure and application of a retinoid, she says, "even the mouse gets the rosy glow that people get."

With JAMA's January publication of the first randomized, double-blind study of topical tretinoin in the treatment of photodamaged human skin, tretinoin made history. An editorial accompanying the research in the normally staid journal was headlined, "At Last! A Medical Treatment for Skin Aging." The new research, wrote Barbara A. Gilchrest of the Boston University School of Medicine, "suggests that a new age has dawned."

Ongoing studies, as yet unpublished, indicate tretinoin continues to reverse many of the effects of skin aging well beyond the 16-week period of that original research.

"What we find in general is that the longer we go, the more improvement we get," says John Voorhees of the University of Michigan Medical Center in Ann Arbor, a coauthor of the JAMA article who has been treating patients with tretinoin for more than 17 months. Even in people who have discontinued use of the drug, he says, "the benefits in general are quite stable for a couple of months."

Despite such enthusiasm, tretinoin – as yet officially approved only for treatment of acne – is still far from attaining broader approval from physicians or government regulators. For one thing, researchers have yet to develop methods to accurately gauge changes in skin elasticity and wrinkling patterns. Without such tools, measures of improvement will always be clouded by subjective interpretation (see box).

Moreover, skin irritation, sometimes severe enough to require a halt in treatment, is a common side effect – at least in the early stages of treatment. Some dermatologists suggest it is by irritation itself that tretinoin imparts the soft, pinkish glow characteristic of its use. But recent research indicates the drug's beneficial effects are most pronounced after the period of at least obvious irritation.

The question remains, then: What exactly is tretinoin *doing* in the skin? Researchers using sensitive helium-neon laser Doppler devices capable of "hearing" blood flow beneath the skin are finding evidence, supported by microscopic studies, that tretinoin stimulates new growth of tiny blood vessels, which in turn may nurture the regeneration of damaged skin cells. Perhaps more important, researchers know that retinoids in general are important gene regulators and play a role in cell growth and differentiation.

Moreover, retinoids can detoxify dangerous oxygen free radicals and can inhibit the degradation of collagen. And scientists have shown that some retinoids can reverse carcinogen-induced precancerous growth, or metaplasia – although it's still unclear, they say, whether tretinoin may help to prevent skin cancer. "There are a lot of questions to be answered," says E. George Thorne, director of clinical research at Ortho Pharmaceutical.

But as Ovid observed nearly 2,000 years ago, unanswered questions are unlikely to discourage demand for a product that at least hints of the qualities of an anti-aging drug. And given the nature of today's health-conscious, media-steeped culture, dermatologists say they are being asked to perform the impossible: to keep patients' skin looking young while these very same patients insist upon languishing on the beach or in tanning salons.

"These people are lost," says Albert Kligman, shaking his head in exaggerated pity. "They think they can ravage their skin in the summer and we'll fix it up in the winter." □