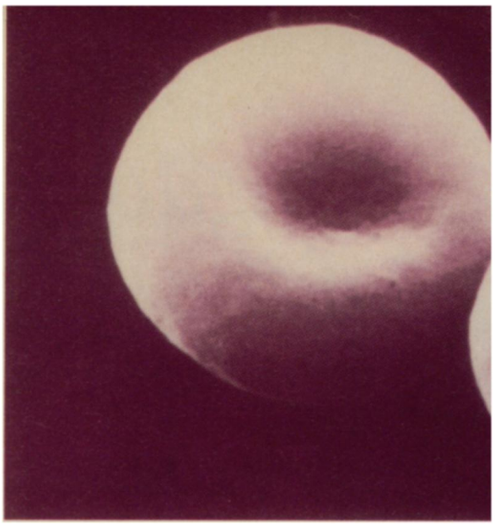
Postponing Red-Cell Retirement



Can aging blood cells get a new lease on life?

By RICK WEISS

Each day, without fanfare, an average adult gives birth to about 200 billion new red blood cells. That amounts to 2.3 million cells squeezing through microscopic, bony "birth canals" every second, emerging from their nurturing birthplace in marrow to make their working debut in the bloodstream. Dutifully they circulate through the body, hauling oxygen atoms to distant tissues in need of the life-giving gas.

Equally impressive and perhaps less appreciated is the daily *disappearance* of another 200 billion red cells. And not just any 200 billion. For the most part, the body picks and chooses from the roughly 25 trillion circulating red cells those 1 percent that have enjoyed long, full lives. Having outlived their usefulness and thus their welcome after 120 days or so on the job, these aging couriers become targets of a poorly understood but impressively efficient biological mechanism that rips them open, recycles their valuable, iron-rich cores and sends the remaining cellular remnants to the liver and kidneys for disposal.

Were it not for this constant scavenging of older red blood cells, says Philip S. Low, a blood chemist from Purdue University at West Lafayette, Ind., "our blood would be thick as concrete in a couple of weeks."

But when the body cleans house, how does it know which red cells to spare and which to sweep away? Do some cells display molecular or behavioral hallmarks of old age that the body recognizes as signs of senescence?

Scientists seek answers to these questions out of a desire to tinker with the mechanisms of cellular death, not just in the blood but throughout the body. Red cells resemble most other cell types in their basic membrane structure but are more easily obtained than many other types. By investigating the tools the body uses to recognize and remove aging blood cells, scientists may eventually gain some control over these biological housekeeping duties elsewhere in the body – perhaps slowing cell death or at least preventing the accelerated removal of healthy cells that occurs in certain diseases.

Even if the work proves applicable only to blood cells, researchers say, such an ability could offer immense benefits to the blood banking industry and to patients who today need frequent transfusions. "The hope," says Low, "is that we can modify or affect the aging process," to prolong the life of red cells either in blood banks or within the body.

But while scientists have made significant progress in understanding red-cell senescence, they have yet to agree upon any one molecular "cause" of cell aging or even a marker that points to cells past their prime, says Margaret R. Clark, a hematologist at the University of California, San Francisco. "We still don't know how to recognize aging red blood cells very well at all," she says. And so far, the markers researchers have proposed as signposts of red-cell senescence reflect "more wishful thinking than hard evidence."

A fundamental problem stalls scientists in their endeavor to understand cell senescence. In order to identify molecular landmarks of the aging process, researchers must get a relatively pure population of aged cells for comparison with younger cells. And in order to pull aside such a population, they must first have a way of identifying them and separating them from their younger counterparts. In short, without markers of aging, it's difficult to find markers of aging.

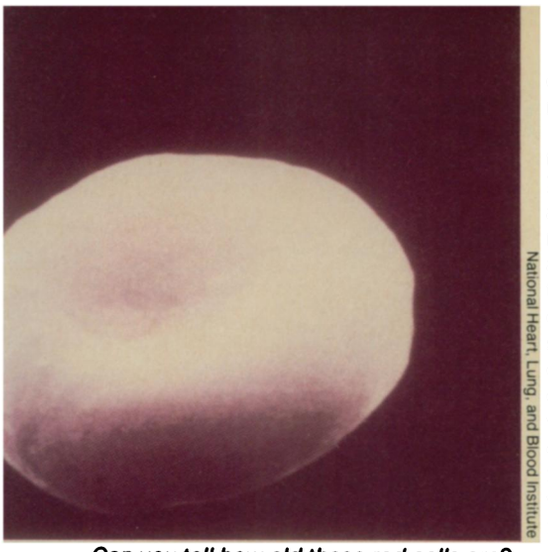
Hematologists have long relied on measures of individual cell density and size to obtain populations of older red blood cells. "The dogma has been that as cells age they become more dense and smaller," says George L. Dale of the Scripps Clinic and Research Foundation in La Jolla, Calif. Upon separating such cells, researchers have identified a handful of alterations that less dense, presumably younger cells seem to lack.

But many scientists think the cell populations culled through the density separation technique reflect more age diversity than previously assumed. So, while the changes observed in dense cells seem intriguing, "the problem is you don't really know if you're dealing with senescent cells," Clark says.

In 1971, hematologists developed an improved means of gathering aged red blood cells. The technique requires researchers to extract blood from large numbers of mice or rats and transfuse it into a smaller number of rodents. The increased blood load suppresses new red-cell production in the recipients. After two weeks, researchers transfuse these cells – none of them younger than two weeks at this point – into other rodents of the same recipient group. Repeating the procedure for about eight weeks – the average lifespan of a rodent red cell – yields a few rodents whose red cells are all very old.

While this system provides reasonably pure populations of aged cells, researchers variously describe it as "very time consuming," "a brute-force technique" and "a pain in the butt."

In search of a better model, Scripps hematologist Dale recently devised what he calls a biotinylation technique for obtaining old red cells. Working with rabbits, Dale separates newborn red cells – easily identified under the microscope because they contain DNA remnants that disappear after the first few days of cell life. He then labels the young cells with



Can you tell how old these red cells are?

biotin, a B vitamin, and infuses them into a test rabbit. A few weeks later he draws some blood from the rabbit and mixes the blood in a chamber that pulls out any biotin-labeled cells. In these cells he looks for changes that have occurred with age. Done at regular intervals, the "bleeds" allow a researcher to observe cell membrane changes and other aging characteristics week after week.

Many researchers praise the new technique, which has already overturned some long-standing theories about red-cell aging. One popular theory — that older red cells gradually become metabolically depleted, losing their ability to generate the cellular fuel called ATP — proved groundless when Dale and his colleagues showed that metabolic activity actually increased with cell age. "We were really surprised by that," he says.

Dale's rabbit studies also showed that the amount of AMP deaminase, an enzyme that degrades a component of ATP, declines in older red cells — a finding since confirmed in human red cells. Although the enzyme decline may provide a useful marker of older cells, it is probably not the essence of cell aging, Dale says. "We don't think it's the critical clock that we're after."

Other researchers have their own theories as to what lies at the heart of red-cell aging. Five years ago, Thomas J. Mueller, Carl W. Jackson and their colleagues at St. Jude Children's Research Hospital in Memphis, Tenn., noted a gradual buildup of a membrane protein called 4.1a — a trend that correlated with increased rigidity of the molecular scaffolding just inside the cell's outer membrane.

The finding led them to propose that old red cells eventually become too rigid to pass through the tiny capillaries in the spleen. "It's really a stressful situation going through the spleen," Jackson says, noting that the organ is relatively acidic and oxygen-poor. "So if you're a rigid old cell and you're about out of gas, then you might not make it through."

Scientists know the spleen serves as the major demolition site for old red cells. But many believe red-cell destruction also involves a complex immunological mechanism in the bloodstream, in which antibodies recognize a molecular feature that develops on the outer membrane of old red cells. According to this theory, says Purdue's Low, antibody-coated red cells get destroyed by white blood cells called macrophages, in much the same way macrophages destroy antibody-coated viruses or bacteria. "A macrophage doesn't give a hill of beans whether the antibody-marked target is a red cell, a virus or a bacterium," Low says.

While the immunological theory is popular, researchers disagree about the nature of the membrane marker that might initiate such a reaction. Marguerite M.B. Kay, a hematologist working at Texas A&M University and at the Olin E. Teague Veterans Center in Temple, Tex., has identified a membrane protein dubbed "band 3" that appears to break down with age to expose a "senescent antigen" recognizable to circulating antibodies (SN: 4/19/86, p.247). More recently, Kay and her colleagues found support for their theory in studies of a patient suffering from a serious, ongoing destruction of young and middle-aged red blood cells — a condition apparently caused, they say, by these cells' abnormal expression of senescent antigen. They describe the case in the August Proceedings of the National Academy of Sciences (Vol.86, No.22).

Others propose different membrane features as the premier targets for antibody-mediated red-cell destruction. Researchers led by Uri Galili of the University of California, San Francisco, have focused on an antibody called anti-Gal. They suggest it binds to a membrane-bound residue, called alpha-galactosyl, that lies hidden until late in a red cell's life.

Still others, including David Aminoff of the University of Michigan at Ann Arbor, contend red-cell aging is accompanied by a loss of sialic acid residues from a membrane protein called glycophorin.

Both the wealth of accumulating data in this field and the lack of consensus among blood researchers are reflected in *The Red Cell* (1989, Alan R. Liss, Inc., George J. Brewer, Ed.), the proceedings of an October 1988 conference at which Kay, Galili, Aminoff, Mueller and others spoke. In the end, Clark told Science News, scientists may find "many different types of changes recognized by different antibodies."

Moreover, says Low, it's likely these various changes in membrane proteins are themselves caused by more fundamental aging processes inside the cells — such as the degradation of hemoglobin, the oxygen-carrying compound in red cells. That hypothesis provides an attractive explanation for the aging mechanism, he says, because it doesn't require the presence of an unmodifiable biological clock. "It allows for removal of any red cell that begins to falter in its major role as an oxygen transporter," he says. "That's the time to get rid of it."

Although the ultimate trigger of red-cell aging and removal remains obscure, scientists express optimism that they will someday characterize these reactions in sufficient detail to manipulate the cellular aging process in useful ways.

"If you can identify why these cells have changed [with age] and if you could come up with some kind of treatment that would prevent them from being cleared, perhaps you could extend their lifespan by a week or two," says Mueller, now at the Indiana University Northwest Center for Medical Education in Gary. Even such a seemingly small extension could bring substantial clinical benefits, he adds. In people recovering from blood loss, for example, transfusions might be unnecessary if physicians could keep old cells at work for the seven days or so it takes for new ones to arrive from the marrow.

An increase in red-cell lifespans could represent a boon to blood banks, Low says. These facilities are perpetually in need of freshly donated cells for the nearly 14 million transfusions performed each year in the United States. If the current five- to six-week shelf life for donated blood could be lengthened by a couple of weeks, "less unused blood would be dumped down the drain and the needs for donated blood would be decreased significantly," he says.

Alternatively, researchers say, transfusion recipients might benefit if technicians could remove most older cells from donor blood. To an already anemic patient, those cells can pose more burden than benefit. Concentrates of young cells, just beginning their life's work, could reduce the frequency of transfusions for patients who need lifelong hematological boosts.

Some people with sickle cell anemia, for instance, today require transfusions on a monthly basis. After years of transfusions, these patients begin to suffer the effects of iron overload because of the body's tendency to hoard the metal. Any reduction in the number of transfusions they require would be beneficial, Jackson says.

Clinical applications must wait, however, until researchers identify definitive markers of cell aging. And in the case of healthy individuals, Low says, a more philosophical question remains: Does the best bodily strategy for a long life involve squeezing the most out of every cell, or are there advantages in hurrying cellular demise to make room for some proverbial new blood? □