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ENVIRONMENTAL HEALTH PERSPECTIVES

MARCH 2006
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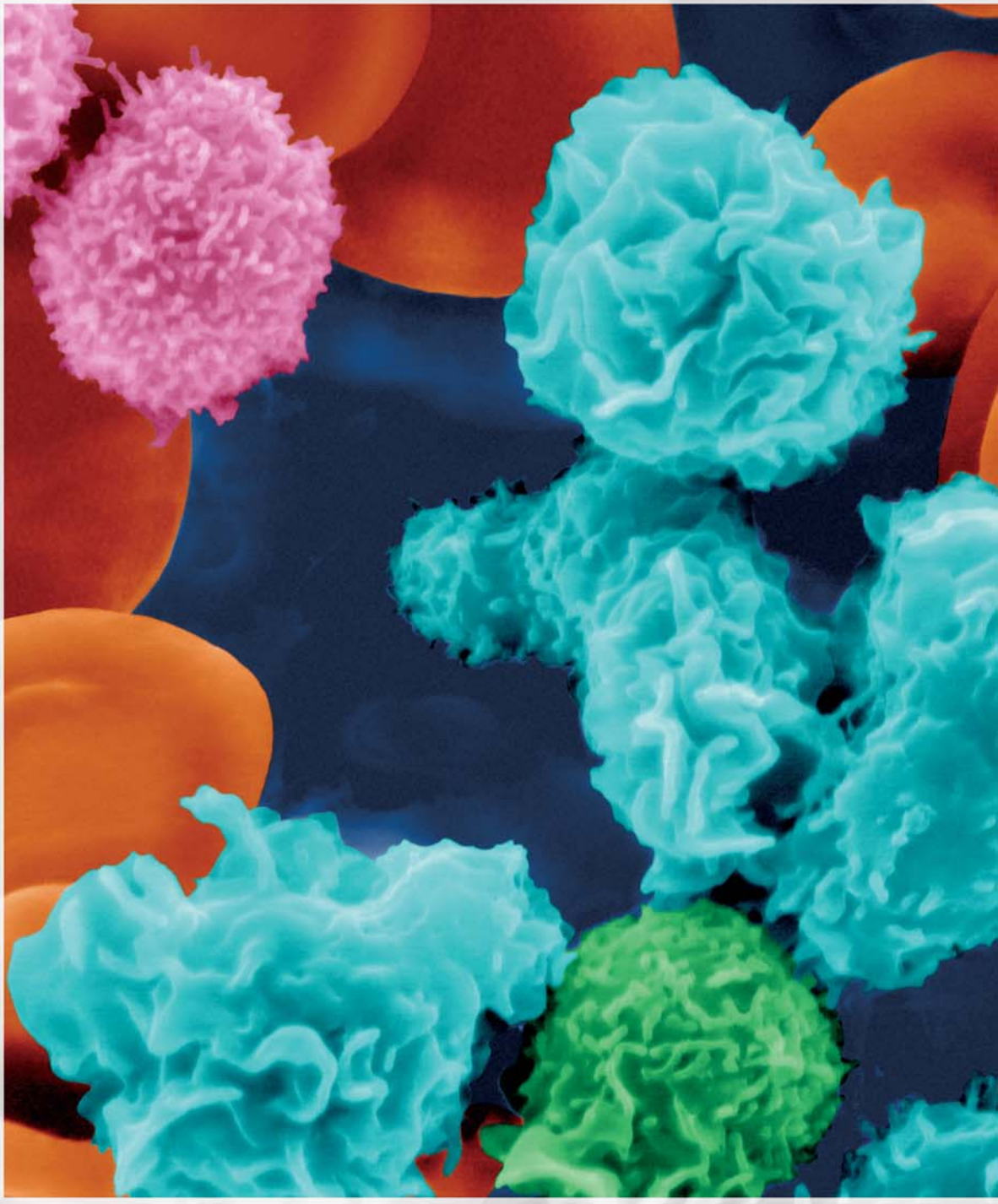

Eyes: Windows to the World

The Case of the Contaminated Maize

Death by Particles

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NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
NIH PUBLICATION 2006-218

STUDENT EDITION

We live in a time when the words *impossible* and *unsolvable* are no longer part of the scientific community's vocabulary. Each day we move closer to trials that will not just minimize the symptoms of disease and injury but eliminate them.
Christopher Reeve
Testimony to U.S. House of Representatives (1999)

SUBSTANCE ABUSE

Resurgence of Teen Inhalant Use

The 2004 Monitoring the Future (MTF) survey showed that inhalant use ("huffing") is rising among American teenage students, particularly 8th graders. The results, released in December 2004, showed that 9.6% of 8th graders used inhalants in 2004, up from 7.7% in 2002 and 8.7% in 2003. Inhalant use was also up slightly among 10th and 12th graders in 2004. Findings from the latest MTF will be released in late December 2005, and researchers are anxious to see if the trend holds.

"These increases are disturbing because they come after a long period of decline in inhalant use by students in all three grades," says Lloyd D. Johnston, a professor at the University of Michigan Institute for Social Research and principal investigator of the MTF since it began in 1975. "We are concerned that the use of this class of drugs may be about to rebound."

Each year, the MTF, which is funded under grants from the National Institute on Drug Abuse (NIDA), asks approximately 50,000 8th-, 10th-, and 12th-grade students in some 400 schools nationwide about their use of drugs, alcohol, and cigarettes. The data gathered are used to help government officials and policy makers identify potential drug problem areas so they can target resources to deal with them.

"We know that inhalant use starts early and that long-term abusers are among the most difficult drug abuse patients to treat," says NIDA director Nora Volkow. "It is critical that research efforts to characterize the behavioral effects of inhalants intensify, so that more effective preventions, interventions, and treatments can be developed." This year, NIDA announced the continuation of a broad-based research initiative begun in 2002 to address the epidemiologic, social, behavioral, cognitive, and neurobiological consequences of inhalant abuse, as well as treatment and prevention.

More than 1,000 readily available products are used as inhalants, and they can potentially kill, according to the Office of National Drug Control Policy (ONDCP). Such products include glue, shoe polish, gasoline, lighter fluid, and the propellants in spray deodorant, hair sprays, and canned whipped cream.

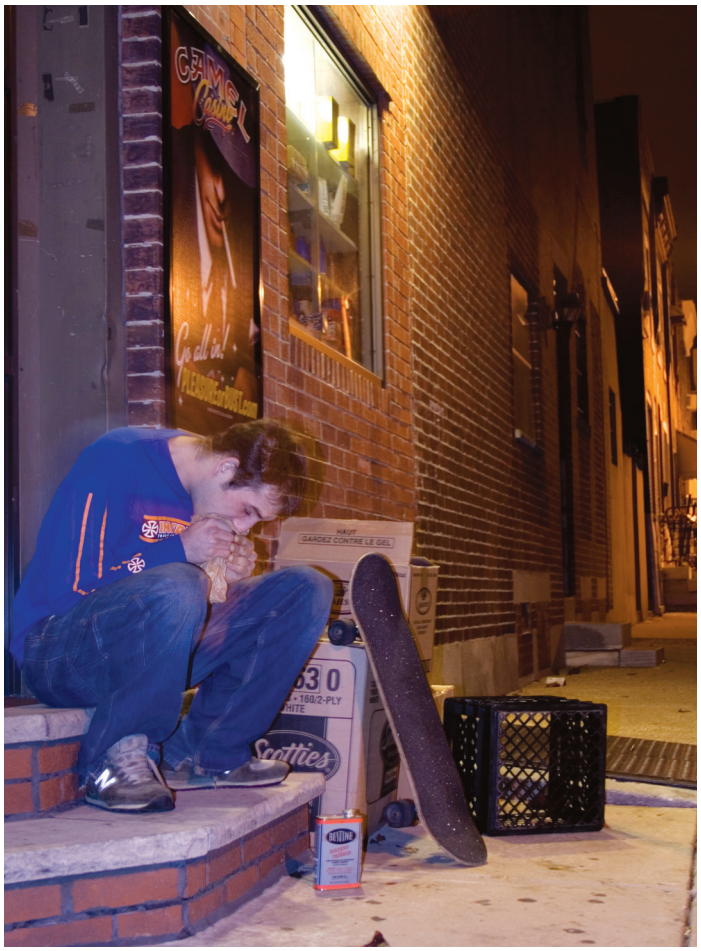
The ONDCP further reports that glue, shoe polish, and toluene-containing products were the most commonly abused inhalants among users aged 12 to 17. According to the American Association of Poison Control Centers, gasoline accounted for the greatest percentage (44%) of reported inhalant deaths between 1996 and 2001, followed by air fresheners (26%) and propane/butane (11%). Other health effects of inhalant use include headache, nausea, vomiting, slurred speech, loss of motor coordination, and wheezing.

The physical and social environment both play a key role in inhalant use, says Harvey Weiss, executive director of the National Inhalant Prevention Coalition. Treatment sometimes requires removing the abuser from the environment in which he or she is abusing inhalants. "We should not view inhalant abuse [simply] as a substance abuse problem," Weiss says. "It's a public health problem, so we need to do more public health outreach to young people."

Sources believe that education is the key to preventing inhalant use from becoming a dangerous fad. When MTF data from the mid-1990s began showing a long-term gradual increase in inhalant use, the Partnership for a Drug-Free America and NIDA mounted an aggressive media campaign about the dangers of inhalants. The next round of MTF data showed a decline in inhalant use and a concurrent increase in young people viewing inhalants' use as risky, but use began climbing again after the media campaign ended.

"Of course, the evidence is circumstantial, but we've seen the same thing happen for so many other drugs," Johnston says. "A drug can have a resurgence in use among young people because of what I call 'generation forgetting'—that is, a new generation of young people comes along that hasn't heard too much about a drug, so it is naïve about the consequences of its use. That begins to change when a public education campaign is launched."

Despite the MTF findings, the U.S. government hasn't yet documented a trend indicating a rise in inhalant use among teenagers, says Terry Zobeck, deputy associate director for policy and budget at the ONDCP. But he adds, "The MTF is respected and well documented. We will be quite concerned if its next survey shows that inhalant use is up for the third year in a row." **–Ron Chepesiuk**



Huffing is up. A new survey shows the practice of inhaling toxic—often deadly—substances is increasing among American teens.

Lori Struss

Autoimmune Disease

Phthalate Linked to Lupus in Mice

No one knows to what degree genetics or environmental agents cause lupus, an autoimmune disorder that affects the skin, joints, and internal organs including the kidneys. However, researchers at Indiana State University may have strengthened the environmental evidence by discovering that phthalates trigger lupus antibodies in a mouse model.

Phthalates are found in adhesives, cosmetics, fragrances, vinyl flooring, polyvinyl chloride pipe, and certain toys and medical supplies. According to a report out of the Centers for Disease Control and Prevention and the National Toxicology Program, published in the October 2000 issue of *EHP*, phthalate exposure is more extensive than previously suspected, especially in women aged 20–40 years. Other studies have pointed to possible links with asthma, rhinitis, and eczema in children as well as altered genital development in male infants. The new lupus findings add to a growing list of potential health effects caused by these chemicals.

In lupus, the immune system loses its ability to tell the difference between foreign substances (antigens) and the body's own cells and tissues. The immune system makes antibodies against the body itself, causing inflammation, tissue injury, and pain. Up to 1.5 million Americans have been diagnosed with lupus, and another 16,000 develop the disease each year, according to the Lupus Foundation of America.

While investigating the gene sequence of a monoclonal antibody used as a marker for tumor growth, biochemist Swapan Ghosh, interim chair of the Life Sciences Department at Indiana State University, noticed that it shared 98% similarity with an antibody protein component (light chain) made by NZB mice, a popular model for autoimmune diseases. In lupus, such antibodies attack DNA in the kidneys, heart, and lungs. The finding, published in the December 2003 issue of *Immunology*, was a surprise: "I was not studying lupus or autoimmune diseases at all," says Ghosh. But he took advantage of the unexpected turn and has launched a series of experiments to further explore the phthalate–lupus connection.

In the latest study, Ghosh and graduate student So-Yon Lim injected four types of mice, including NZB mice, with di-(2-ethylhexyl) phthalate, or DEHP. Initially, all the mice generated antiphthalate antibodies, but only the lupus-prone NZB mice developed nephritis, which led to kidney failure and early death. The other mice initially produced antiphthalate antibodies, but the antibodies were counteracted by CD8+ suppressor T cells, which prevented kidney damage. "There's something different about the immune systems of NZB mice [that makes them more susceptible to phthalates]," says Ghosh. The details of the investigation are reported in the August 2005 issue of the *Journal of Autoimmunity*.

Although the phthalate–lupus connection has been observed only in mice, "many things found in the mouse immune system have proven to be true in humans," says Ghosh. On the other hand, "not everything seen in a mouse model reflects what happens in humans," cautions Betty Diamond, chief of rheumatology at Columbia University.

Although Ghosh's results are far from applicable to humans, they do raise several questions for future studies on the potential phthalate–lupus link in people. Do lupus patients have high levels of antiphthalate antibodies? Ghosh plans to screen lupus patients and healthy people in the future to find out. Does exposure to phthalates increase the risk for lupus? He plans to explore this, too, by measuring blood levels in workers exposed to phthalates in the plastics manufacturing industry. Lupus is five times more common in women than men. Might this be because women use more phthalate-containing cosmetics and perfumes than men do?

The American Chemistry Council (ACC), an industry trade group, has criticized Ghosh's study because he combined DEHP to proteins like bovine serum albumin. "The attached proteins may cause autoimmune and allergic responses," says Marian Stanley, director of the ACC's Phthalate Esters Panel. Ghosh counters, "We also studied DEHP not complexed to a protein, and it evoked an anti-DNA response." He explains that he attached a main metabolite of DEHP to proteins because some studies have suggested that phthalate metabolites show an affinity for albumin in the body.

So far, exposure to ultraviolet light is the only environmental factor that has been clearly linked to lupus in genetically susceptible patients. As lupus researchers continue to investigate other environmental causes, "we need to be open-minded, but not jump to conclusions," Diamond says. **–Carol Potera**

Fly the Environmentally Friendly Skies

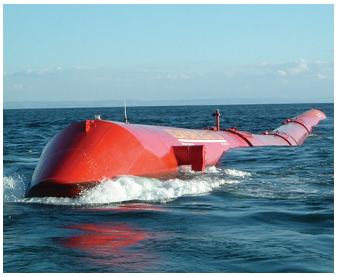
In June 2005, the British airline industry unveiled a 15-year initiative to make itself more environmentally friendly. The industry wants to improve its fuel efficiency, reduce perceived external noise, and lower carbon dioxide emissions on new planes by 50% and nitrogen oxide emissions by 80%. Also planned are ways to give travelers information on the amount of fuel used and pollutants emitted on routes that they travel. The industry may also prohibit foreign carriers from flying older, more-polluting aircraft into the United Kingdom.

A Loan for Colombia

In June 2005 the World Bank announced it was granting a $150 million loan to Colombia to help that nation integrate sustainability principles into its environmental programs and policies and meet the UN Millennium Development Goals, including halving the number of people without adequate water and sanitation facilities. The monies are earmarked for three areas: development of a framework for planning and monitoring the progress toward meeting the UN goals; increased interinstitutional cooperation and public participation in environmental decision making; and development of laws and policies related to air and water quality, solid waste management, and environmental licensing. Bank officials hope the work financed by the loan will also decrease child mortality rates related to respiratory and diarrheal diseases.

Wave Power in the Works

Just off the northern coast of Portugal is the site of the world's first commercial wave-generated electric plant. The contract was signed in May 2005 for the $9.6 million project, under which three wave energy converters will be built at the site. The long, hinged converters move with the flow of tidal currents, pumping fluid to hydraulic motors that drive generators.



The wave power plant is expected to provide electricity for more than 1,500 Portuguese households while displacing more than 6,000 metric tons of carbon dioxide produced each year by conventional power plants. If this first phase proves successful, 30 additional wave converters will be ordered by the end of 2006.

Top to bottom: David R. Frazier Photolibrary/Alamy; Ocean Power Delivery

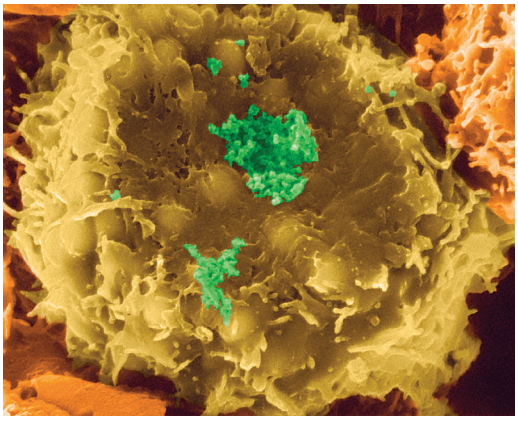
INFECTIOUS DISEASE

New Human Retroviruses

Retroviruses called human T-lymphotropic viruses (HTLVs) are found in two types—HTLV-1 and HTLV-2—in people all over the world. Genetic evidence suggests that they crossed into humans from simian T-lymphotropic viruses (STLVs) and that each type, plus various subtypes, have crossed independently. Now, two more types of HTLV have been found in humans living in central Africa.

At least 22 million humans are infected with HTLV-1 or HTLV-2, and the viruses are endemic in several areas. About 2–5% of those infected with HTLV-1 develop adult T cell leukemia. HTLV-1 also causes a neurologic disease called tropical spastic paraparesis/HTLV-1 associated myelopathy. HTLV-2 is less pathogenic but is thought to cause similar neurologic illnesses and increase susceptibility to opportunistic infection.

William Switzer, a researcher at the Centers for Disease Control and Prevention, and his colleagues sequenced HTLV strains from a high-risk population: people in Cameroon who reported contact with nonhuman primate tissues through hunting and butchering or keeping primate pets. The study uncovered many previously unknown subtypes of HTLV-1, most with known correlates in nonhuman primates. The team also found that two people carried previously unknown HTLV types. One, HTLV-3, is similar to the nonhuman primate virus STLV-3. The other, HTLV-4, is genetically different from any known virus in humans or other primates. The findings appear in the 31 May 2005 issue of the *Proceedings of the National Academy of Sciences*.



Cross-species predator. A T lymphocyte infected with HTLV-1 (green), which causes a type of leukemia. Such viruses are believed to have crossed to humans from simians.

Because HTLV-4 is so divergent from other HTLVs, this virus may have evolved in humans over quite some time, Switzer says. It's possible, though, that primates are infected with an equally divergent simian version that just hasn't been found yet. "We're screening primates in that same area to see if we can answer that question," Switzer says.

A group led by Antoine Gessain, head of the Epidemiology and Physiopathology of Oncogenic Viruses Unit at the Pasteur Institute, also recently found a subtype of HTLV-3 in a human, but it's somewhat different from the subtype Switzer and his colleagues found, which suggests "another example of multiple independent, cross-species transmission events," Switzer says. The HTLV-3 strain Gessain found is extremely similar to a strain reported in the red-capped mangabey, which suggests that it crossed to humans very recently, Switzer says. Gessain's findings were published 9 May 2005 in *Retrovirology*.

The current dogma surrounding retroviruses is that cross-species transmission is rare, but finding so many near-identical strains between humans and nonhuman primates suggests this is not a rare event. Benign retroviruses probably cross from nonhuman primates to humans frequently, but we don't notice them because we don't get sick, says Bernard Poiesz, a professor of medicine at SUNY Upstate Medical University. "But every once in a while," he says, "one of them will jump and we may not handle it so well." **–Melissa Lee Phillips**

ECOLOGICAL CHANGE

Life Lessons

"All global environmental change eventually ends up as a human health problem," said Eric Chivian, director of the Harvard Center for Health and the Global Environment, opening the August 2005 First International Conference on Health and Biodiversity in Galway, Ireland. Speaker after speaker showed how careless disregard for the environment and its variety of life forms squanders potential new medicines, endangers our food security, and exposes us to new risks of infectious disease.

Many frequently prescribed drugs are derived from or patterned after compounds in natural sources, Chivian noted. For example, ziconotide—a pain killer 1,000 times more powerful than morphine—comes from marine cone snails that inhabit narrow ranges in coral reefs and thus are increasingly endangered by coral bleaching, mostly from global warming. How many other useful species are lost without our ever recognizing their potential?

Species loss may also mean the loss of valuable models for medical research, said Chivian. Black bears, which hibernate for several months over the winter without losing bone mass, could provide a clue to the cause of osteoporosis, an enormous public health problem. But bear populations in many parts of the world are threatened by habitat destruction and overhunting.

Discussion of sustainable food systems for developing countries focused on promoting the use of indigenous plants. In Lebanon, where diets are high in bread and refined grains but low in fruits, vegetables, and fish, a quarter of the children are overweight and a third of the women of childbearing age are anemic. Malek Batal, a nutrition professor at the American University of Beirut, is exploring how wild plants such as fennel, mint, and salsify have the potential to increase diversity of nutrient intake and food security in poor communities. He found that wild plants offer antioxidants, flavonoids, fiber, iron, calcium, and many other nutrients. Being easily accessible, easy to use, and palatable, they also contribute to food security.

Interfering with ecosystems can have dire consequences for biodiversity, as conservation biologist Diana Bell of the University of East Anglia explained: when the South American myxoma virus was introduced into Europe in the 1950s to control rabbit populations, it contributed to the collapse of a species-rich ecosystem in which the rabbit was the keystone prey for more than 45 predators. Bell also identified the illegal trade in wildlife (especially small carnivores) in Southeast Asia as a dual threat to human health (as the origin of the SARS coronavirus) and massive species loss in this "biodiversity hot spot." She believes an interdisciplinary approach involving ecologists, microbiologists, medical specialists, and others will best advance research in the twin fields of human health and species loss.

The time to address biodiversity loss is now, speakers agreed. As Chivian said, "We are in deep, deep trouble with what we are doing to life on Earth. . . . We are tampering with the life support systems of the Earth in ways that we barely understand." **–Dorothy Bonn**

Dennis Kunkel Microscopy

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National Eye Institute

The National Eye Institute (NEI) is the primary institute of the NIH for supporting and conducting research on preventing, diagnosing, and treating eye diseases and other vision disorders. Currently the NEI oversees approximately 1,600 research projects at more than 250 institutions in addition to the research ongoing at its own facilities in Bethesda, Maryland. The institute also works to translate research findings into clinical applications and to raise public awareness about eye and vision problems. The NEI uses its website, located at http://www.nei.nih.gov/, to help disseminate information about its many programs.

The What's New section provides links to newly released NEI-funded research and other topics of interest to those in the field. The more in-depth News and Events section includes press releases, clinical alerts for professionals, information on meetings and special events, and a list of official statements and reports on vision.

The Health Information page links to information on 21 eye diseases and disorders. There is also a section on basic eye anatomy with diagrams of the eye, links to glossaries of eye terminology, a collection of eye care resources, NEI information provided in Spanish, and a way to order NEI materials online. The collection of eye care resources consists of an eye health organizations database; a page of frequently asked questions about clinical trials and how they are conducted; and tips on finding an eye care professional, procuring financial assistance for eye care, and talking to doctors about eye health.

National Eye Institute
NATIONAL INSTITUTES OF HEALTH

More information on NEI clinical trials is available on the Research Funding page and through the Clinical Studies Database. The Research Funding page has information on grant and funding opportunities for researchers, news on staff appointments, updates on grants and funding policies, and overviews of councils and workshops of interest to NEI researchers, among other resources. The Clinical Studies Database provides a list of all ongoing and completed NEI-supported studies. This section also includes study results and lists of journal articles that have been generated by the research, as well as a list of NEI studies that are currently enrolling participants. Site visitors can search the database for studies under six topic areas or by keyword, study location, age of study participants, patient recruitment status, or study status.

The Education Programs page offers overviews of NEI outreach activities. Through the National Eye Health Education Program, the NEI conducts large-scale public and professional educational activities in partnership with national organizations. Specialty initiatives within this program focus on diabetic eye disease, glaucoma, low vision (when everyday tasks become difficult to do even with corrective lenses, medicine, or surgery), and educating Spanish-speaking Americans about eye and vision problems. VISION is a teaching supplement for grades 4 through 8 that is available for download at no charge. This 16-page guide helps teachers plan lessons about how the eye works, eye problems, and eye safety. The supplement was developed in cooperation with the Association for Research in Vision and Ophthalmology. THE EYE SITE is an NEI-sponsored exhibit that travels to shopping malls around the United States to educate the public about low vision, vision rehabilitation services, and vision adaptive devices, as well as about the NEI itself. The exhibit features five colorful kiosks and an interactive multimedia program. Another exhibit, VISION, educates visitors to science museums about how vision works and about how researchers are working to develop ways to protect our eyes from disease and developmental problems. **–Erin E. Dooley**

Baytril Gets the Boot from Bird Farms

Amidst calls from doctors and public health advocates, the FDA has banned the use of the antibiotic Baytril in poultry. The FDA is also reviewing requests to ban the use of other drugs given to animals. Although Perdue Farms and other producers stopped using Baytril before the July 2005 ban, an industry spokesman said alternative drugs are not as effective in dealing with respiratory illnesses in mass-produced poultry.

The ban is intended to stop the increase of drug-resistant strains of foodborne *Campylobacter*. *Campylobacter* infection causes abdominal symptoms and fever, and is one of the most common bacterial causes of diarrheal illness in the United States. According to the FDA, 20% of human *Campylobacter* infections involve the resistant strain.

WHO Knows About Radon?

The WHO has launched the International Radon Project to educate the public about the hazards of this chemically inert, radioactive gas that occurs naturally in soils and rocks around the world. The project will include a database of average radon levels in member nations, radon action levels, and mitigation measures, among other information. The WHO has also published a new fact sheet on radon and cancer as part of the project.

Radon may cause 6–15% of lung cancer cases, and moderate exposure may increase the risk of lung cancer in smokers by 25 times. Radon exposure in homes varies according to a home's location, ventilation, and presence of exterior cracks and openings.

From Carpet to Kilowatts

Each year some 4.7 billion pounds of carpet are taken to U.S. dumps, taking up almost 1% of the country's landfill space. Now Shaw Industries, the world's largest carpet maker, has opened a $10 million power plant that is fueled by the 16,000 tons of scrap the company turns out annually as well as by 6,000 tons of sawdust produced by wood flooring manufacturing. The new plant powers one of the company's main factories, and should save the company $2.5 million in fuel oil each year. The plant engineers say the process emits about the same amount of pollution as natural gas.

Top to bottom: Creatas; NEI; Ingram Publishing/Alamy

Abdiction/Addiction Connection

Sometimes in science, as in politics, connections arise that may at first glance appear to be strange bedfellows. That might be the natural first impression of a potential association between chemical intolerance and addiction. But although the conditions are manifested by behaviors that appear to be polar opposites—substance avoidance (or abdiction, as some are beginning to call it) by the chemically intolerant, and compulsive substance use by the addicted—there is evidence to suggest that, biologically, they may actually have much in common.

That was the concept behind "Addiction and Chemical Intolerance: A Shared Etiology?" This conference, held 19–20 September 2005 in Research Triangle Park, North Carolina, was the first scientific meeting to be cosponsored by the NIEHS and the National Institute on Alcohol Abuse and Alcoholism (NIAAA). It was also the first time researchers from the fields of environmental health and addiction convened to explore common ground and potential collaborations.

"The idea of hosting a conference on chemical intolerance and addiction stems from a long history of individual physicians' reporting observations on patients that looked like addiction to chemicals, foods, caffeine, or alcoholic beverages," explained conference chair Claudia Miller, a professor and researcher in environmental medicine at The University of Texas Health Science Center at San Antonio. "There is a striking resemblance between the symptoms and responses to substances reported by chemically intolerant patients and individuals addicted to drugs or alcohol."

Firm numbers on addiction and chemical intolerance are hard to come by, in part because both conditions often go undiagnosed. Approximately 67% of all Americans drink alcohol, yet 90% of the alcohol is consumed by only 30% of the population, said NIAAA director Ting-Kai Li in his keynote address. In the latter half of 2003 (the most recent year for which figures are available), there were 627,923 drug-related emergency room visits in the United States, according to the Drug Abuse Warning Network of the U.S. Substance Abuse and Mental Health Services Administration. As for chemical intolerance, epidemiologic figures compiled and reported at the meeting by William Meggs, a professor of emergency medicine at East Carolina University, suggest the prevalence of the condition (self-reported) to be approximately 12% of the U.S. population, with approximately 4% self-reporting as "seriously affected."

Miller contends that addiction and chemical intolerance represent divergent physiologic responses to a shared underlying disease mechanism she calls toxicant-induced loss of tolerance (TILT). In TILT, a chemical exposure—either acute or chronic and low-level—initiates sensitization to even small amounts of structurally diverse chemicals found in foods, drugs, alcoholic and caffeinated beverages, pesticides, mold toxins and other elements of indoor air, implanted devices, solvents, cleaning chemicals, and more. Thereafter, when affected individuals are exposed to everyday "triggering" substances such as foods, traffic exhaust, or fragrances, they report multisystem symptoms including headache, nausea, difficulty breathing, muscle spasms, and rashes. The fact that different people exhibit different constellations of symptoms has made it difficult to conduct epidemiologic studies or arrive at a case definition, Miller says. In the past, these difficulties have led some observers to speculate that chemical intolerance is psychogenic in origin.

As she outlined in her presentation to the approximately 120 attendees, Miller postulates that the TILT mechanism can lead to either abdiction or addiction, with both behaviors intended to avoid unpleasant withdrawal symptoms. She further proposes that TILT may underlie a wide variety of chronic diseases that are increasing in prevalence worldwide, such as asthma, autism, chronic fatigue syndrome, fibromyalgia, and depression. (She described these proposals in depth in an article in the January 2001 issue of *Addiction*.)

Parallel Paths

Whether or not chemical intolerance and addiction are flip sides of the same coin, it is clear that researchers in the two fields have much to learn from each other. Li said, "Some people become alcohol-dependent and then they recover because the environmental risks have been removed; there's a gene–environment interaction. I think it's

Joseph Tart/EHP

true also for chemical intolerances. It's an environmentally induced condition, and when you remove the environmental risk, the person may still be genetically high-risk, but without the environmental component they can then recover."

As things stand today, however, there are no easy answers for the chemically intolerant. Environmental epidemiologist Howard Hu daily perceives the need for more research in his role as a clinician at the Harvard School of Public Health. "Our environmental medicine clinic has several hundred patients who have this disorder, and we have not made any progress in ways to evaluate and manage them that has led to any sustainable improvements in their condition," he said. "So we really appreciate the need for good research that will shed light on the biology of the disorder and allow us to devise methods to manage and treat it."

Hu felt that the conference was a good step forward in helping to define a research agenda. "Some of the approaches to chemical addiction and alcoholism [research] have provided a roadmap of where the chemical intolerance research needs to go, in terms of understanding genetic susceptibility and the molecular changes that might be the mechanism of how the intolerance phenotype develops," he said. One role model for progress described by Miller might be the Japanese government, which has established several environmentally controlled medical units (EMUs) in hospitals for the research, diagnosis, and treatment of chemical intolerance. To date, there is no comparable facility in the United States.

One speaker called attention to "tantalizing morsels" of convergence that have emerged between chemical intolerance and addiction. For example, it appears all but certain that genetic susceptibility plays an important role in both conditions, and one of the most compelling ideas to emerge was the possibility that susceptibility to both conditions may arise from polymorphisms in the same genomic neighborhood—genes including *CYP2D6*, *PON1*, and others that are known to regulate the metabolism of exogenous agents such as drugs and pesticides. *PON1* is involved in the detoxification of organophosphate pesticides; *CYP2D6* functions in the metabolism of structurally diverse substances that affect the central nervous system, including various classes of antidepressants, amphetamines, codeine, and neurotoxicants. The question of whether variant alleles of these genes give rise to the abdiction and addiction phenotypes is a primary target for investigation in the future.

For now, case–control study results presented by researchers Cornelia Baines and Gail McKeown-Eyssen of the University of Toronto (which were published in the October 2004 *International Journal of Epidemiology*) clearly show an elevated risk for chemical intolerance associated with variations in the enzymatic metabolism genes *CYP2D6*, *PON1*, and *NAT2*. A gene–gene interaction detected between *CYP2D6* and *NAT2* suggested that rapid metabolism alleles in both genes may confer as much as an 18-fold elevated risk for chemical intolerance. These findings point toward a biologic basis for the condition.

Brain imaging studies presented at the conference by Hu, Marc Potenza of the Yale University School of Medicine, and Leonid Bunegin of The University of Texas Health Science Center at San Antonio showed striking similarities between chemically intolerant patients and addicted individuals in terms of the neural regions involved and the types of activation detected. Many signs point to the mesolimbic system, where the activity of neurotransmitters such as dopamine is regulated. Among individuals who are genetically susceptible to either chemical intolerance or addiction, the homeostasis of the brain's reward system may be upset or perhaps changed permanently by exposures to certain drugs or chemicals. Thus, although the outcomes of addiction and abdiction may be polar opposites, the underlying causes and mechanisms may prove to be very similar.

> There is a striking resemblance between the symptoms and responses to substances reported by chemically intolerant patients and individuals addicted to drugs or alcohol.
>
> –Claudia Miller
> The University of Texas Health Science Center at San Antonio

You Say Tomato . . .

Differences in nomenclature often pose a challenge and require reconciliation when two fields begin to work together. As one conference presenter waggishly put it, "Scientists would rather use each other's toothbrushes than use each other's terminology."

Perhaps the best example of varying terminology arose as speakers from both fields presented some of the leading hypotheses in each field. In chemical intolerance, researchers refer to "initiation" (the exposure that leads to the development of intolerance) and "triggering" (subsequent exposures resulting in symptoms); in addiction research, scientists refer to neurologic "sensitization" to a substance leading to "amplification" of its effects.

According to Miller, future research may show that neurologic sensitization also explains initiation and triggering. "Perhaps the processes [underlying addiction and chemical intolerance] are one and the same, but we don't know that quite yet," she says. "Eventually, once the biology has been worked out, the terminology may reconcile, clarifying the links between the two fields. It was one of the most striking parallels to emerge from the meeting."

Another impediment discussed during the proceedings is the longstanding struggle to precisely define phenotypes of chemical intolerance for research purposes. Single-minded focus on this difficulty in the past has been the excuse for doing no research, said Miller, who added that facilities like Japan's EMUs could be used to assess individual responses in the absence of any consensus on case definitions or phenotypes. "Just as there is no single case definition or phenotype that encompasses all forms of drug and alcohol addiction, there is no single case definition that can be applied to all forms of abdiction, because we are dealing with a general mechanism for new classes of diseases that have varied manifestations," she explained.

Establishing a chemical intolerance phenotype or case definition is further complicated by a phenomenon called "masking." Underlying chemical or food triggers may be masked by overlapping symptoms resulting from simultaneous or sequential exposures to other foods or chemicals, from addiction to caffeine, alcohol, or tobacco, and from varying degrees of habituation to triggering substances. For example, Miller wrote in her *Addiction* paper, "[i]f an individual is sensitive to many different substances, then the effects of everyday exposures to chemicals, foods, or drugs may overlap, producing a confusing array of symptoms. The individual would feel sick most of the time, but the

effect of any single exposure would not be apparent to either the individual or his physicians." Masking therefore confounds diagnosis and treatment because clinicians tend to address patients' overt symptoms without discovering the underlying intolerances, much less the initiating exposures that led to illness in the first place.

The lack of phenotypes may also hamper the application of systems biology to the study of chemical intolerance. Systems biology integrates tools from genomics, proteomics, metabolomics, and informatics to detect and validate novel biomarkers of disease. "Without a phenotype, it's difficult to move to the next level," said William Slikker, Jr., deputy center director for research at the National Center for Toxicological Research. First, he suggested, we still need to define phenotypes in a way in which they can be systematically examined. "Once that is done," he said, "then I can see setting hypotheses that can be tested using the systems biology approach."

At the same time, the availability of a research EMU—the equivalent of a detox unit for alcohol or drug withdrawal—would provide a unique tool for examining individuals' genetic and protein expression before and after removal of chemical and food triggers and before and after specific challenges, said Miller. "Just as systems biology will enable researchers to understand individual responses to complex environments, the EMU is a tool that [would allow] us to identify the responses of individuals to a wide variety of exposures," she said.

Miller said the approaches are completely compatible and complementary. "A clear advantage of the EMU is that it can be done now—well before sophisticated genomic and proteomic approaches become widely available—and begin to benefit patients with a wide variety of environmentally induced illnesses."

The Road Ahead

NIEHS deputy director Samuel Wilson, who opened the meeting, agreed that the future of the field depends largely on researchers' ability to carefully identify researchable questions. "It's going to be up to the scientists writing the proposals or bringing the problems forward to figure out experimental themes or researchable problems that they can make a case for, and then work up and make solid discoveries on," he said. "There's no substitute for having quantitative traits to look at—quantitative biochemical markers or biomarkers that can be related with exposure and with these very complex behavioral phenotypes."

Wilson added that only when the molecular science embedded in the pathophysiology and biology of chemical intolerance and addiction is uncovered will the extent of overlap between the two conditions be established.

Several attendees expressed great interest in pursuing collaborative projects with colleagues from the other field, and many were optimistic that the conference would ultimately result in cross-institute initiatives between the NIEHS and NIAAA. For environmental health researchers, addiction has long been a blind spot; in addiction research, the same is true for environmental exposures. With greater interactions between the two fields, both may achieve a clearer view of these conditions and the road to health. **–Ernie Hood**

Effecting change in Southwest communities. Krystyn Yepa (right) is one of several nursing interns working with Navajo and Sioux communities to reduce exposures and increase knowledge through a program of the University of New Mexico Center for Environmental Health Sciences.

Left to right: Michael Warnock/iStockphoto; University of New Mexico COEP

BEYOND THE BENCH

Nurses Adapt to Changing Health Care Climate

With increased emphasis being placed on the importance of environmental health comes the need for an expanded variety of environmental health care practitioners. Nursing is one of several professions that are augmenting and advancing the capabilities of their practitioners to meet this need. Now the Community Outreach and Education Program (COEP) of the University of New Mexico's Center for Environmental Health Sciences has partnered with the New Mexico Environment Department to create an environmental health nursing internship as a component of its outreach program.

In cooperation with the university College of Nursing, the partners recruit nursing students who are interested in implementing environmental health care initiatives in the surrounding community. For four years, the program has taken nursing students beyond the traditional curriculum and shown them firsthand how interaction with environmental factors affects human health. The program also gives students valuable experience in taking measures to abate hazardous exposures in communities.

The interns are currently working on a number of projects that will affect different community members. They are helping to develop surveys and compiling data for a project investigating uranium exposure and subsequent kidney damage among the Navajo Nation, whose members live near and work in uranium

mines. They are gathering and assembling materials for a community education and survey project on mercury in surface waters and other environmental health concerns among the Cheyenne River Sioux Tribe. They are helping COEP staff write and field-test an integrated environmental health curriculum on diabetes for middle school students. And in a fourth project, they are helping to develop a best-practices manual for applying farm waste fertilizer to croplands in a way that minimizes human exposure to aerosolized waste.

The local ties of some of the nursing interns have enhanced the program's role as an effective community advocate. Intern Krystyn Yepa is a Native American from the Pueblo of Jemez who became interested in environmental health nursing because she wanted to understand some of the health effects in her tribe resulting from exposure to different pollutants in the environment. "Learning about the different environmental health problems that exist in New Mexico has given me the willpower to finish nursing school to ultimately achieve my goal of improving the health and lifestyles of my people by placing importance on the environment," she says.

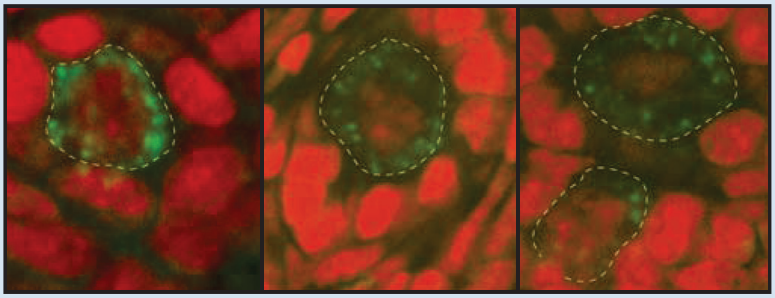
Yepa explains further, "As a Native American, I was raised to respect and appreciate the environment, and working at the COEP has only strengthened my values related to the environment." She also credits the internship with teaching her important assessment tools that lay emphasis on the environment when completing a health history on patients, something a traditional nursing internship would not likely provide.

"The nursing interns have been an incredible asset to our COEP," says staff member Stefani Hines. "It is a mutually beneficial situation on many levels—the student nurses gather valuable real-world experiences in environmental health; the nursing school has access to additional, unique placements for their students; we have additional support for projects, which helps make them better; and the communities we work with benefit from the students' efforts as well."

Dedicated, enthusiastic environmental health nursing interns will only continue to play an important role in advancing the COEP mission. One project currently in development will help community members get involved in city and county zoning processes, which will both encourage a healthy community mindset and minimize exposures to pollutants. **–Tanya Tillett**

Headliners | Reproduction

NIEHS-Supported Research



Johnson et al.

Oocyte Generation in Adult Mice

Johnson J, Bagley J, Skaznik-Wikiel M, Lee H-J, Adams GB, Niikura Y, Tschudy KS, Tilly TC, Cortes ML, Forkert R, Spitzer T, Iacomini J, Scadden DT, Tilly JL. 2005. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell 122:303–315.

The theory that female mammals are born with a finite number of germ cells (oocytes) has been accepted as an unquestionable truth for over 50 years. Recent research has challenged this accepted dogma by showing that mice and flies can produce oocytes and follicles during puberty and adulthood. Now NIEHS grantee Jonathan L. Tilly and colleagues at the Harvard Medical School have shown that adult mice can produce large numbers of new oocytes in a short period of time, providing additional evidence to challenge the accepted belief of a fixed complement of oocytes at birth. The Harvard researchers also discovered a source of germline stem cells in the bone marrow.

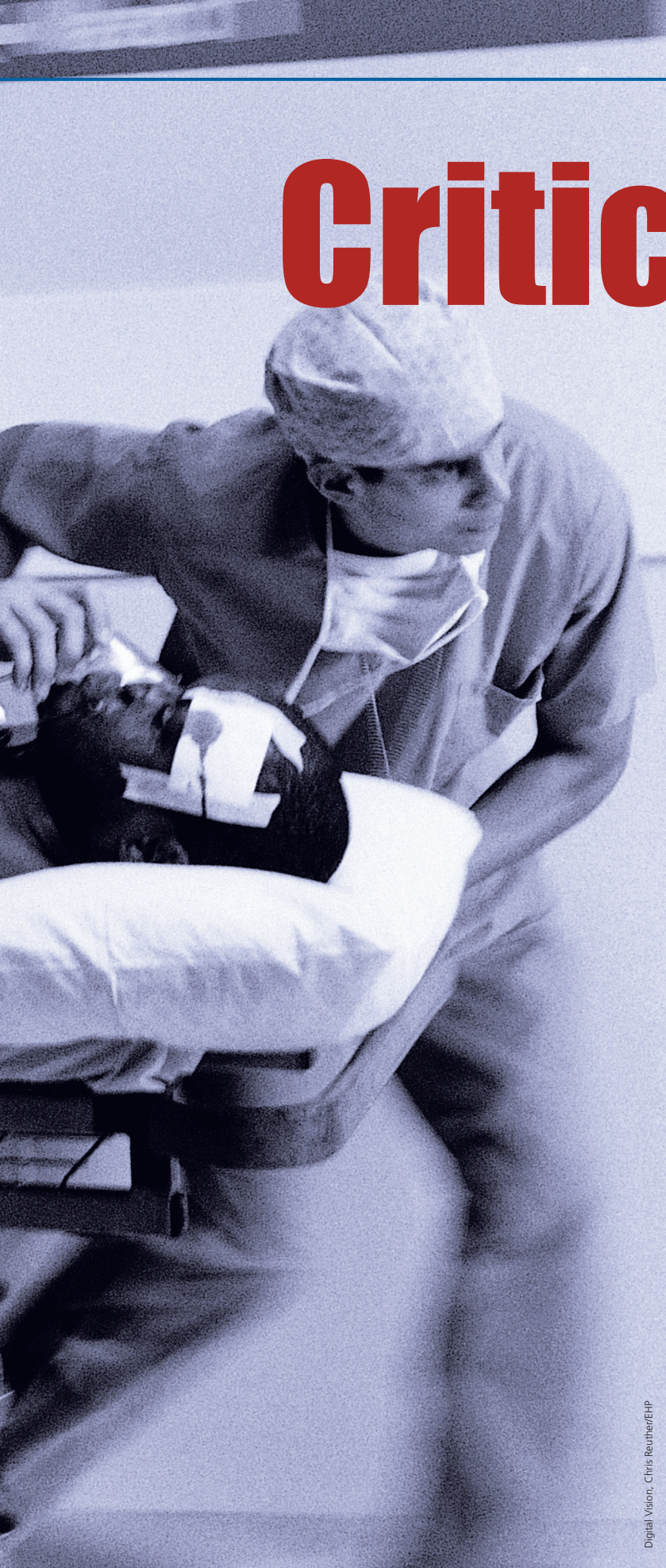
Oocytes are found in the ovaries surrounded by somatic cells in structures known as follicles. Only a small fraction of follicles actually reach ovulation, producing an egg capable of being fertilized. Conventional wisdom hold that in humans, only about 30,000 of an original pool of about 1 million oocytes present at birth are still present at puberty, and this number is thought to gradually decline throughout adulthood until the complete loss of oocytes at around age 50 stimulates menopause. Acceptance of the concept that adult mammals can continue to produce oocytes has been slow, likely due to the lack of direct evidence of the existence of mammalian female germline stem cells.

The Harvard team conducted gene expression analysis and bone marrow transplantation studies on mice that had been sterilized through chemotherapy. Within 24 hours of treatment, follicles were regrowing in the animals' ovaries. By 2 months after treatment, there was no difference between the treated animals and controls. In other studies, mice whose bone marrow was destroyed with chemotherapeutic agents were injected with peripheral blood from transgenic animals with germline cells expressing green fluorescent protein. Oocytes found in the test animals' ovaries within 30 hours of treatment also expressed the fluorescent protein.

The researchers have not yet determined whether oocytes derived from germline stem cells can undergo fertilization and subsequently develop into viable offspring. However, the results do prove that bone marrow and peripheral blood are sources of germline stem cells and can sustain oocyte production into adulthood. If adult oocyte production is also possible in humans, it could have major implications for the treatment of infertility and other disorders such as osteoporosis, although much additional research is needed before this potential can be realized. **–Jerry Phelps**

Critical Care

Applying Genomics to Inflammation Outcomes



Digital Vision, Chris Reuther/EHP

What do gunshot wounds, burns, heart attacks, arthritis, asthma, and cancer all share in common? Apart from inflicting misery, these conditions—and others too—involve inflammation, an immune response to injury and infection that normally protects, but sometimes endangers or kills patients. Caused by immune cells accumulating at a site of injury, inflammation typically guards against infection and speeds recovery; it is a critical process and, per se, does not cause disease. But unchecked inflammation that spreads or fails to subside poses chronic and acute health risks for millions of people. Asthma patients, for instance, can't breathe because inflammatory compounds cause airway linings to swell and mucus to spread in the lungs. Inflammation also exacerbates cancer, scientists believe, by facilitating the proliferation of abnormal cells. An acute condition called sepsis—caused when infection or inflammation spills into the bloodstream—produces organ failure and shock in critically ill patients. Up to 215,000 Americans die from sepsis every year, according to the National Institute of General Medical Sciences. Worldwide, sepsis is estimated to kill 1,400 people each day, according to a consensus document published in the June 1992 issue of *Chest*.

In light of its implications, inflammation has become one of the hottest areas in biomedical research. J. Perren Cobb, a professor of surgery and genetics at Washington University in St. Louis, says a wide array of medical specialties stand to benefit from these investigations. "Inflammation is a major unifying syndrome, the investigation of which provides opportunities for multidisciplinary convergence," he explains. "Studies of inflammation cut across all the domains at the NIH; it's a fundamental process in human biology that ties everything together."

Growing evidence suggests that genetic factors drive key aspects of an individual's inflammatory outcome. Scientists studying inflammation are trying to identify the genes that drive inflammation as well as biomarkers from throughout the course of

inflammation. Stephen Chanock, who heads the Section on Genomic Variation in the Pediatric Oncology Branch at the National Cancer Institute, emphasizes that the current critical care orientation of this research has broad multidisciplinary implications that extend to environmental health. "Injuries represent the ultimate gene–environment interactions," he explains. "Usually environmental health focuses on chronic exposures, but in this case we're studying environmental insults that are more dangerous and intense. So, the 'environment' in environmental health isn't just about pollution, it's also experiential. We're developing practical methods for looking at inflammation that will ultimately be applied to larger public health issues."

Toward Better Knowledge of Inflammation

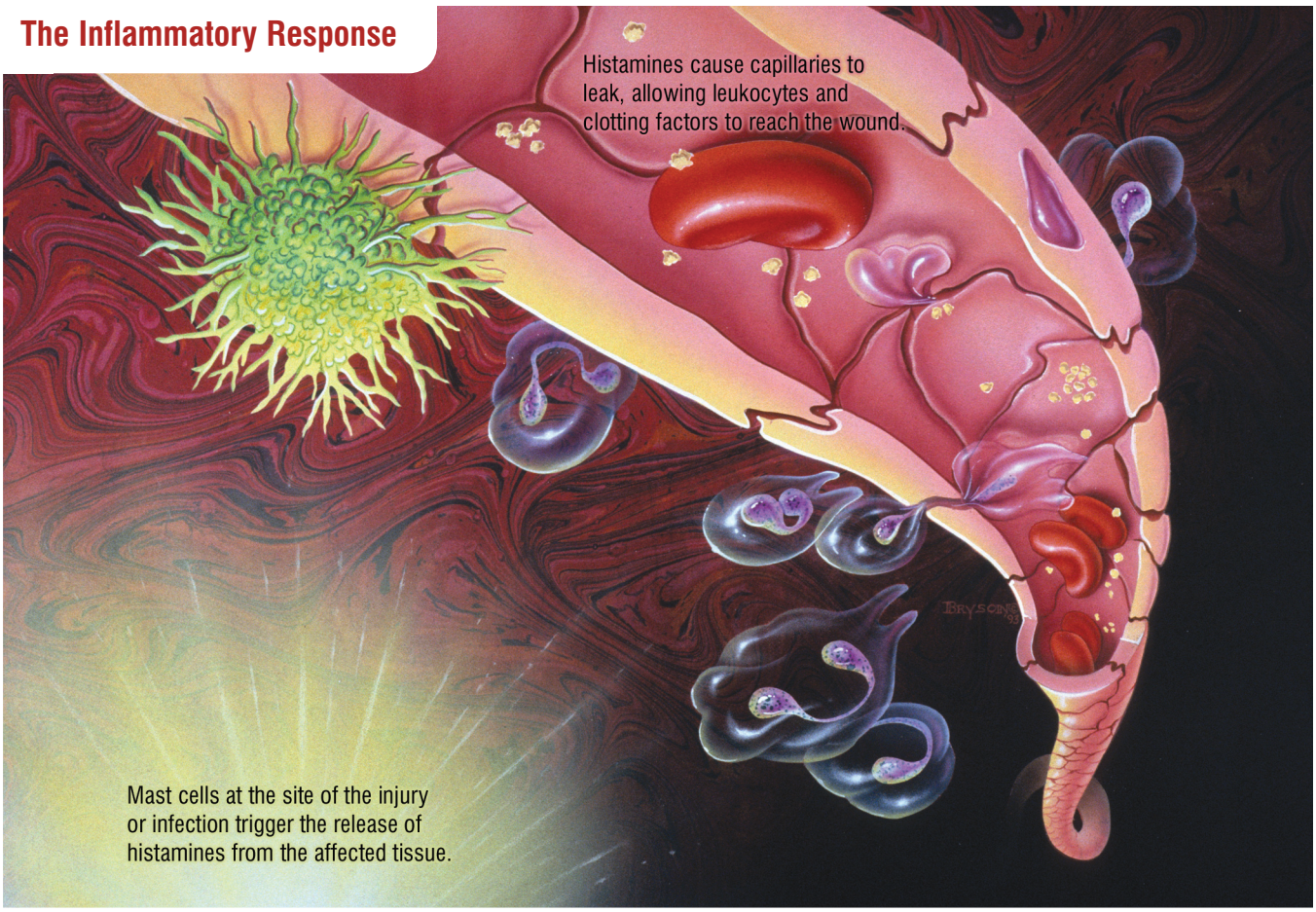
Today, genomics defines the cutting edge of inflammation research. Genomic studies, in addition to their proteomic and metabolomic cousins, aim to resolve an age-old mystery: namely, why some patients recover readily from inflammation while others suffer and die from it. The current research emphasis focuses on critical care, particularly of trauma and burn patients, who face the lethal dangers of septic complications. Ideally, new gene-based discoveries will provide diagnostic biomarkers to predict who among these patients will react poorly to inflammation and why. If doctors could reliably predict this outcome in advance, they might tailor antibiotics and other treatment options to a patient's own inflammatory system, potentially saving lives.

Better knowledge of inflammation biology could also spawn new treatment options, Cobb says. The newest drug for sepsis—an Eli Lilly and Company product called Xigris that came on the market in 2001—helps some patients, but its cost is exorbitant: nearly $7,000 per course of treatment. What's more, the drug reduces the risk of death by just 6% and can produce side effects such as excessive bleeding.

Among the numerous programs moving inflammation research forward is an effort funded by a National Institute of General Medical Sciences "glue grant," so named because it "glues together" multidisciplinary efforts to tackle biomedical questions beyond the means of any one research group. This program, called Inflammation and the Host Response to Injury, strives to determine why patients can have dramatically different outcomes after traumatic injuries and burns. Headed by Ronald Tompkins, a professor of surgery at Harvard Medical School and chief of Massachusetts General Hospital's Burn Service, the program uses genomic and proteomic methods to study inflammation at 22 clinical centers located throughout the country. A total of $37 million was made available for the program's first five years.

When the Inflammation and Host Response to Injury program was launched in 2001, its leaders decided to create a broad research infrastructure with uniform protocols as a first priority. "One of our first challenges was to develop guidelines, not just for the sample collection and analysis, but also for patient management," says Lyle Moldawer, a glue grant recipient and professor of surgery at the University of Florida College of Medicine. "We recognized that all the funded centers have different protocols for the immediate care of trauma and burn patients, and we were concerned that those differences in early

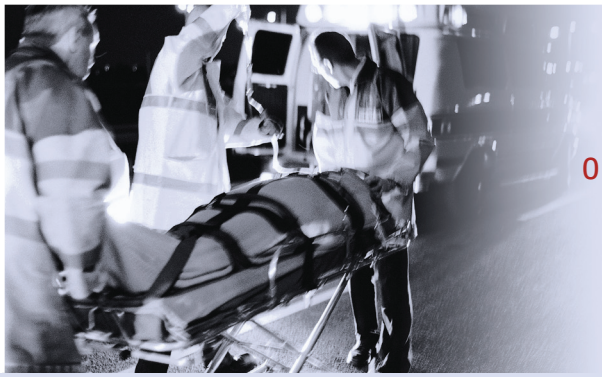
The Inflammatory Response



Bryson/Custom Medical Stock Photo

management might contribute to gene expression changes."

Tompkins says creating a uniform infrastructure for the program was like building a highway. "We needed the gas stations, the on-ramps, the off-ramps," he says. "No one had ever tried to introduce this technology into critical care medicine before." With standard operating procedures in place and the program now in its fourth year, scientists have begun to address a subsequent challenge: extracting useful knowledge from the reams of genomic data flowing out of the program's 22 clinics.



> Injuries represent the ultimate gene–environment interactions. Usually environmental health focuses on chronic exposures, but in this case we're studying environmental insults that are more dangerous and intense.
>
> –Stephen Chanock
> National Cancer Institute

At the same time the glue grant program was gearing up, Cobb, senior investigator Anthony Suffredini of the NIH Critical Care Medicine Department, and Robert Danner, who heads the Infectious Diseases Section in the same department, created the Consortium for Expression Profile Studies in Sepsis specifically to identify the needs of those applying genomic methods to critical care. The consortium hosted four meetings throughout the country before evolving into the NIH Functional Genomics of Critical Illness and Injury Symposia series, which now provides a forum where glue grant recipients and others discuss research progress and results. The most recent symposium, hosted by the NIH at its Bethesda campus on 21–22 April 2005, was attended by scientists from 10 countries, all seeking to advance genomics in inflammation research.

An Inflammation Primer

Once triggered, inflammation proceeds similarly whether caused by pollutants, pathogens, trauma, radiation, or burns. Localized mast cells in affected tissues produce histamine, a chemical mediator that dilates blood vessels at the site of injury, producing redness and heat. Histamine also renders blood vessels permeable, so leukocytes (white blood cells) can reach the injury. Leukocytes are attracted to the injury site by chemotactic proteins known as chemokines, which are secreted by endothelial cells of the blood vessels.

Leukocytes originate in bone marrow and include diverse cell types, such as neutrophils, eosinophils, basophils, monocytes, lymphocytes, and macrophages. Neutrophils arrive at the affected area first. These remarkable cells roam the body and kill pathogens on demand with a toxic blend of free radicals and protein-chewing enzymes that destroy bacterial cell walls. Monocytes engulf cellular debris and mature into macrophages, which are larger leukocytes that consume entire bacteria. These cells also secrete a variety of cytokines that recruit and activate other cell types. Lymphocytes are divided in two broad classes—B cells and T cells—each with different roles. B cells, once activated, make antibodies that attack foreign substances, while T cells kill infected cells directly.

Chemical mediators released by leukocytes during inflammation come in many varieties. Cytokines, for instance, help to regulate inflammation, whereas interleukins regulate T cell activity and produce systemic effects such as fever.

Normally, the whole inflammation process is self-limited and short-lived; leukocytes disperse after dispensing with infectious agents, and inflammation dies down within hours or days. Problems crop up when the response persists or spreads systemically, damaging and killing normal tissues in the process. Chronic inflammation can persist for years, causing illnesses that end with the suffix "-itis," such as bronchitis, arthritis, and bursitis. Systemic inflammation—sepsis being one variety—occurs when cytokines reach the bloodstream and spread through the body, damaging organs far from the initial injury's source.

Candidate Genes

No one knows precisely what happens when inflammation goes awry. Years of immunology research have implicated hundreds of genes in abnormal inflammation, but the evidence linking them to particular outcomes is weak. Of these genes, the one coding for C-reactive protein (CRP), an acute-phase molecule whose levels shoot up during systemic inflammation, is perhaps the best known. High CRP levels are prognosticators for heart disease and stroke (which are both linked to inflammation), but its role in these conditions remains unclear. Another well-known gene—tumor necrosis factor–alpha (TNF-α)—codes for a pro-inflammatory cytokine that normally regulates leukocyte and endothelial cell activity, in addition to other functions.

By the 1990s, however, candidate gene studies had yet to produce clinical benefits for inflammation. Suffredini says scientists at the time were extremely frustrated with the lack of progress. "People were throwing up their hands and feeling [painted] into corners," he says.

A turning point emerged at the turn of the millennium, when a rough draft of the human genome and the advent of microarrays made it possible to assess the expression of thousands of genes simultaneously. "The analogy is that for years, we'd been working on the ground to see how candidate genes interact," Cobb explains. "But microarrays allowed us to look down at the genome from twenty thousand feet, so to speak, and that has enabled us to model much broader interactions."

With these tools, scientists could search for entirely new genes and molecular pathways involved in disease processes. Cancer researchers were among the first to exploit the technology for clinical aims, Suffredini says, inspiring their counterparts in critical care to do the same. Thus, inflammation research entered a new phase of gene discovery that drives much of the progress in the field today. Scientists are now investigating a variation in the promoter region of TNF-α (the region that initiates protein production after binding transcription factors) that might contribute to sepsis.

Digital Vision

While cancer genomics inspired similar efforts in critical care, both specialties operate under vastly different research settings. For one thing, cancer patients typically have the time and awareness to provide informed consent for blood and tissue sampling. In addition, the cohorts tend to be large and matched for age, sex, treatment history, and other parameters that can influence genomic profiles. Trauma and burn patients, on the other hand, are rushed—often unconscious—into the emergency room or intensive care unit, where live-saving treatment is the first priority. In this frenetic environment, informed consent is difficult to secure, and research sampling becomes a secondary concern.

Moreover, cancer and trauma induce totally different types of gene expression—whereas tumors typically produce localized, stable expression profiles corresponding to small portions of the genome, critical injuries trigger enormous genomic changes that affect all tissues and shift rapidly over time. Temporal factors are extremely important in critical care sampling because they have a tremendous influence on the gene profile; a sample taken 15 minutes after injury will be vastly different than one taken several hours later.

Inflammation is a major unifying syndrome, the investigation of which provides opportunities for multidisciplinary convergence. . . . It's a fundamental process in human biology that ties everything together.

–J. Perren Cobb
Washington University in St. Louis

Into the Data

According to Tompkins, investigators with the glue grant program chose to investigate normal and abnormal inflammation trajectories sequentially, each in five-year increments. Genomic and proteomic data for the normal trajectory—compiled using samples from trauma and burn patients who recovered uneventfully—are now being analyzed.

At the same time, program scientists augmented the clinical research with additional genomewide expression studies of leukocytes sampled from healthy volunteers dosed intravenously with bacterial endotoxin. These studies—which induced low-level systemic inflammation that permitted validation of sample processing protocols—enabled scientists to compare baseline and inflammatory genomic changes at varying time points. Patients weren't harmed by the experiments, and all responses returned to normal within 24 hours.

The results, published in the 31 August 2005 issue of *Nature*, showed how complex inflammatory networks really are—between 3,000 and 5,000 genes, up to 20% of the entire genome, were activated, according to Moldawer, one of the study's authors. "The research revealed that the magnitude of the changes was much larger than we anticipated," he says. "We expected to see up-regulation of stress-related genes during the acute phase, but much to our surprise, the diversity of the changes was much greater than we thought it would be."

Many of those changes, Moldawer adds, were seen in genes involved in mitochondrial energy transfer, protein synthesis, and antigen recognition—in short, biological processes that enable leukocytes to become more efficient antimicrobial agents, he says. Preliminary analyses suggested the magnitude and nature of the endotoxin response shared some similarities with the response seen in real patients. At press time, the clinical data from actual patient cohorts were still being assessed.

Although the amounts of genomic data may be computationally daunting, recent evidence from another study suggests efforts to distinguish good inflammatory outcomes from bad might have promise. This study, published in the 29 March 2005 *Proceedings of the National Academy of Sciences*, made several key discoveries. First, hospitalization and repeated sampling had only a modest effect on gene expression in healthy volunteers. Thus, the experience of being hospitalized (with its enforced bed rest and defined nutritional intake) is unlikely to influence gene expression in ways that undermine the detection of signature profiles for specific inflammatory outcomes. Second, the researchers showed that gene expression differences in whole-blood leukocytes drawn from severe trauma patients could be divided into injury-specific patterns. Taken together, says coauthor Tompkins, the findings indicate that expression profiling may yield "low-hanging fruit" in the form of highly correlated data.

Linking Sepsis-Related Genes to Biology

Meanwhile, researchers in Germany have shown that subsets of genes can be linked directly to sepsis. Among these researchers is Trinad Chakraborty, who directs the Institute of Medical Microbiology at Justus-Liebig University. Chakraborty is completing a study of genomic factors contributing to sepsis in patients with multiple trauma or pneumonia. The study—part of a broader effort to understand why patient outcomes differ after similar injuries and illnesses—involved screening up to 20,000 genes in peripheral blood during a 14-day post-injury period. The effort, conducted in 185 patients, found 690 genes whose expression appears to correlate with sepsis. In future research, Chakraborty plans to look for single-nucleotide polymorphisms within candidate genes that predispose the sepsis phenotype, and to identify protein-based biomarkers for diagnostic use.

But Chakraborty adds that computational challenges are a serious holdup. "When we started the research, getting the microarrays to be sufficiently robust was the bottleneck," he says. "Now we've resolved that problem, and bioinformatics is the bottleneck." He and his colleagues hope to trim the 690 genes to a lesser population of 25 or so. "Then we could develop an algorithm that recognizes a profile within that smaller set of genes to indicate whether you have a likelihood of sepsis or not."

U.S. scientists have also correlated genes with sepsis and used these findings to suggest a preliminary mechanism for its lethality. Led by Hector Wong, who directs the Division of Critical Care Medicine at Cincinnati Children's Hospital Medical Center, the scientists used microarrays to compare gene profiles between children who survived sepsis and those who died from it. Children respond uniquely to sepsis in that their fatality

Photodisc

rates are much lower than those of adults—roughly 10% compared to 30% among the latter, says Chanock.

Wong suspects that children respond better to sepsis in part because they have fewer comorbidities such as diabetes and heart disease (a status that is changing somewhat with rising childhood obesity). But he further suspects genetic factors underlie important biological differences that improve their outcomes, though at this point he can't say how.

In recent studies presented at the April symposium, Wong found that among nonsurviving children, six genes coding for metallothionein—a protein that binds zinc and removes it from the bloodstream—appeared to be highly expressed. These findings led him to a hypothesis: if severely septic children had high blood metallothionein levels, he proposed, then their blood zinc levels might be correspondingly low. "And in fact, that turned out to be true," he says.

Another interesting finding was that the profiles showed altered expression patterns for a host of proteins that either depend on zinc or take part in zinc homeostasis. "So there's a lot of biology there to look at," Wong says. "We don't know how or whether zinc is involved; there's very little information out there about the effects of acute zinc deficiency. I find it hard to believe the foundation for sepsis is zinc, but . . . I think it can be tested." After considering this position further, Wong adds that this is how high-throughput investigations are useful: they suggest biological mechanisms that scientists can explore further in the laboratory.

Future Needs

Today, a genomic research culture is slowly seeping into the front lines of care for the critically ill and injured. But establishing that culture isn't easy—emergency room and intensive care unit settings challenge researchers in many ways. Issues like informed consent for study participation and repeated intrusive blood sampling to assess temporal changes in the genome are difficult to manage, Tompkins says. Ideally, new technologies will reduce sample volume requirements, lessen the amount of time required for microarray analysis (which now averages 24 hours), and reduce microarray costs to the extent that they can be used routinely in the clinic.

Chakraborty adds that microarray platforms need to accommodate sample degradation too. As it stands now, he says, RNA in blood samples drawn in the emergency room has a higher degradation potential than RNA in samples drawn from the more controlled environment of a research laboratory. "The platforms need to become more robust," Chakraborty says. "That way, if the quality of the RNA drops to fifty or seventy percent rather than a hundred percent, we would still be able to get meaningful results." Researchers with the glue grant program are also seeking to set up guidelines for standardized research procedures that will help lessen the potential for sample degradation.

We need to do a better job of educating people about the importance of this process.

–J. Perren Cobb
Washington University in St. Louis

Inflammation genomics also poses enormous computational challenges. Studies that lack sufficient statistical rigor are a persistent problem, Cobb says—emergency room and intensive care unit cohorts tend to be smaller than optimal, and patients come in after the trauma has occurred so they can't serve as their own controls. At the same time, lists of inflammation-specific genes identified during microarray experiments need to be incorporated into biological models that describe their molecular interactions. Bioinformatic research and associated databases are continually advancing to meet these needs, however, and collaborations among research groups both within the United States and abroad are helping to drive the science forward.

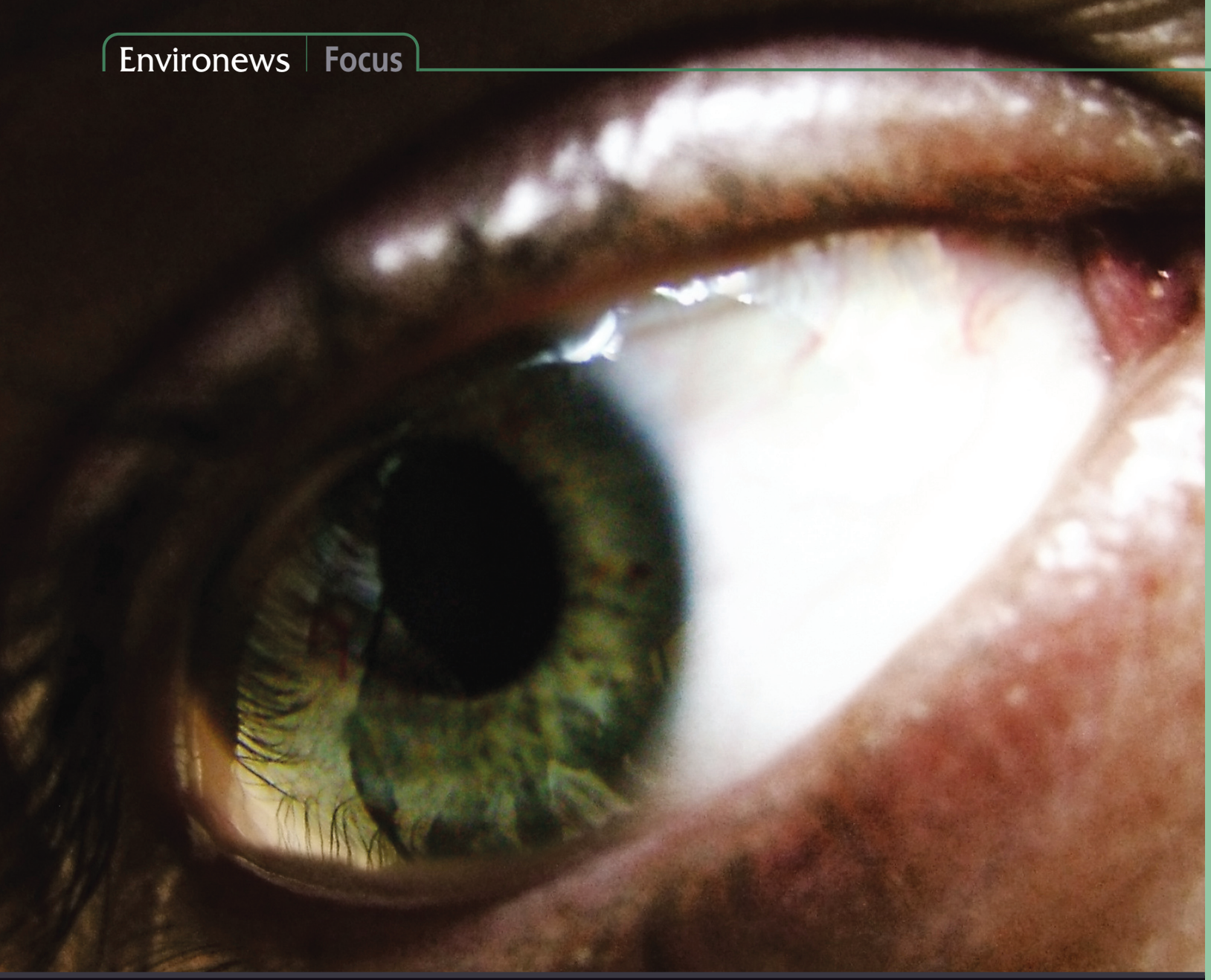
Cobb emphasizes that despite its broad public health impact, inflammation research has yet to achieve the same public awareness as that of cancer or heart disease. "We need to do a better job of educating people about the importance of this process," he says. This means reaching other scientists as well as the public, whose concerns often drive research funding.

In the meantime, genomic methods have generated incremental advances in our understanding of inflammation. Scientists have barely scratched the surface of its vast complexity, but perhaps in the not-too-distant future, patients will reap the benefits of their efforts.

Charles W. Schmidt

Small advances. Fatality rates from sepsis are much lower in children than adults, so much may be learned from how children's bodies deal with inflammation.

Top to bottom: Digital Vision; Photodisc



Focusing on Vision

Through an Environmental Lens

Our eyes are our window to the world, but for many people the view becomes dim or even darkens entirely due to visual impairment. Although the full impact of the environment on sight is unknown and significant gaps remain in our understanding of vision disorders, many reports have shown that low vision and blindness can be directly or indirectly related to environmental exposures.

Vision is described in terms of visual acuity and field of vision. Visual acuity is a measure of how well an individual sees compared with someone with normal sight—for example, a person with 20/60 vision must be within 20 feet of an object to see it as clearly as a normal-sighted person at 60 feet—and a normal field of vision is 160 to 170 degrees.

The World Health Organization (WHO) estimates that approximately 124 million people have low vision, which it defines as visual acuity between 20/60 and 20/400 with the best possible correction or a visual field of 20 degrees or less. Another 37 million people meet the WHO definition for blindness, which is visual acuity that cannot be corrected to better than 20/400 or a visual field of 10 degrees or less. An analysis published in the April 2004 *Archives of Ophthalmology* by the Eye Disease Prevalence Research Group, a consortium representing several institutions, indicates that low vision or blindness affects 3.3 million Americans over age 40 and predicts that this figure may be as high as 5.5 million by 2020. (In this study, low vision was defined as visual acuity between 20/40 and 20/200 with best correction, while blindness was defined as visual acuity of 20/200 or worse with best correction.)

According to the WHO, the rising trend is likely to be seen globally as well. An expanding population explains some of the increase, but more critically, the fastest-growing population sector comprises people older than 50. Worldwide, more than 80% of people who are blind are 50 or older, although they represent only 19% of the world's population. Gender is also significantly associated with visual impairment. Women represent two-thirds of those with blinding eye disease, even after controlling for women's longer life spans.

Risk also varies by race, ethnicity, and world region. Socioeconomic development often predicts regional prevalence of a disorder. Of the approximately 1.4 million children with blindness in the world, about 75% live in high-poverty areas in Asia and Africa. Among children in high- and middle-income countries, optic nerve defects, other neurological problems, and retinopathy of prematurity (a consequence of incomplete eye development) are the most common causes of blindness.

Developing nations are disproportionately affected due in large part to the burdens associated with poverty: lack of clean water and sanitation, limited or nonexistent health care, and malnutrition. Among children in low-income countries, vision problems arise mostly from complications of measles or rubella, nutritional deficiency, improper or inadequate treatment, and eye infections in the first days of life. In Tibet, an area with one of the highest prevalences of cataract, a lack of vitamin A is compounded by exposure to high-altitude ultraviolet (UV) light, soot and pathogens from indoor burning of coal and yak dung, and a dusty, windy environment. As a result, 10.9% of the total Tibetan population suffers visual impairment.

Cataract

The primary insult to the eye is age, and one very common result of aging is cataract, in which the lens acquires color and may also become clouded or opaque. "If you live long enough, you will get cataract," says Roger Truscott, an associate professor and senior research fellow at the Save Sight Institute at the University of Sydney in Australia. Non–age-related cataract arises from specific mutations in membrane proteins, injury, toxic or infectious exposures, and diabetes. Family studies have shown that genetics has a role in heritable cataract and may influence the development of age-related cataract, although no specific genes have been identified.

Cataract ranks as the leading cause of blindness and low vision worldwide. The WHO estimates that nearly 48% of global blindness arises from cataract. Cataract removal is one of the most common surgeries in the United States, with approximately 1 million operations performed each year. In developing countries, cataract can mean permanent blindness because sight-restoring treatment is unavailable or unaffordable for many people.

Among the suspected environmental contributors to age-related cataract are UV light exposure, cigarette smoking, and a diet low in antioxidants. Natalie Kurinij, program director of the NEI Vision Research Program, cites results published over the years from the Chesapeake Bay Waterman Study and the Salisbury Eye Evaluation Project as supportive evidence of the risk presented by UV exposure. "We're fairly confident that sunlight exposure plays a role," says Kurinij.

That role seems to be linked to oxidative damage, which follows the generation of free radicals. The same sort of mechanism is suspected with

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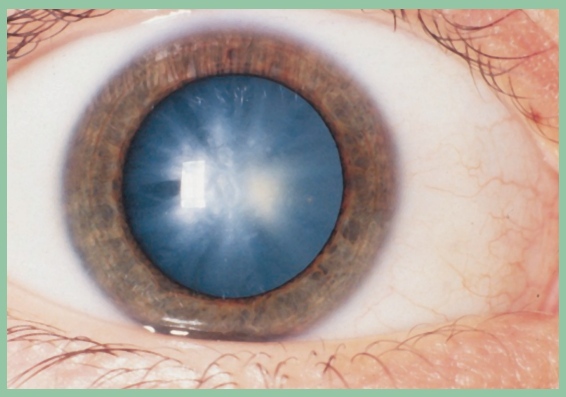
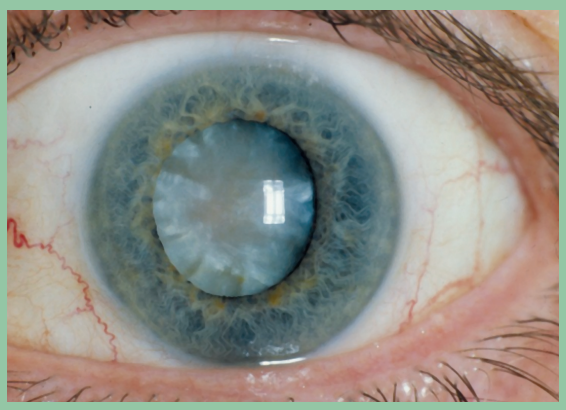
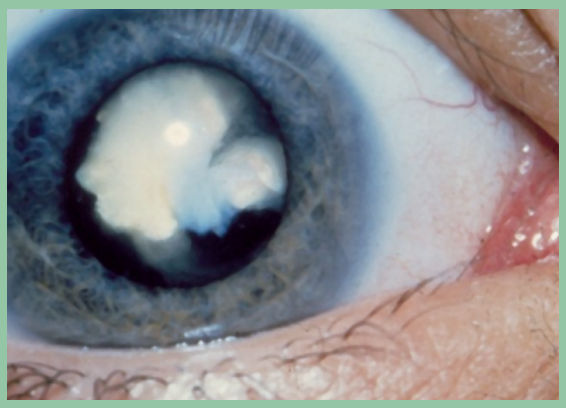
cataract risk associated with smoking. Smoking generates free radicals throughout the body, and those may be responsible for lens damage. The precise mechanisms by which oxidative damage to the lens occurs are still being investigated.

The relationship between free radicals and lens damage may not be direct, however. "The epidemiological data are surprisingly weak when you think that our eyes are exposed for about half of our lifetime, and it seems to make sense that a transparent tissue, designed to transmit light, should suffer ultraviolet damage," says Truscott. The lens possesses a good UV filter system, but it decreases with age. Further, the lens's ability to maintain low oxygen levels within its center also seems to diminish, and the lens thus becomes more susceptible to oxidative damage. Due to these changes in the lens, the compounds that serve as UV filters may bind to lens proteins, which then become more sensitive to UV radiation–induced damage.







Clouding our vision. Cataract, the leading cause of blindness worldwide, arises from a combination of factors including genetics, age, and environmental exposures. Though largely treatable, poor access to health care leaves many around the world to suffer. (Top to bottom) An acute sudden-onset cortical cataract in a person with type 1 diabetes; a hypermature age-related cataract; a white congenital cataract.

A diet rich in free radical–scavenging antioxidants might be protective, but proof is lacking. "Vitamin data are not convincing with regard to cataract, but [vitamins] E and C could be useful. There's still much more to know," says Truscott. Kurinij agrees that support for nutrition's role varies. "There have been conflicting reports from observational studies regarding the role of antioxidant nutrients and the development of cataract," she says. "Any potential effect of antioxidant nutrients on cataract will probably depend on the nutritional status of the population to begin with."

To illustrate the complexity of researching dietary effects on vision, Kurinij compares randomized clinical trial results from the NEI's Age-Related Eye Disease Study to cataract studies conducted in Linxian, China. In the NEI study, high-dose antioxidant and zinc supplements over a six-year period was not associated with lens opacities in a healthy, well-nourished U.S. population. However, in the relatively nutritionally deprived Linxian subjects a multivitamin supplement or a supplement with riboflavin and niacin was associated with fewer cases of cataract.

Oxidative damage also figures in research conducted by Debra Schaumberg, a clinical associate scientist at Schepens Eye Research Institute and an assistant professor of medicine and ophthalmology at Harvard Medical School. Schaumberg and her colleagues reported in the April 1999 issue of the *Annals of Epidemiology* that people with higher blood levels of C-reactive protein, an indicator of systemic inflammation, had a higher incidence of cataract. "This was really the first time that anyone had shown that systemic inflammation, with no clinically detectable inflammation in the eye, increased the risk of cataract," says Schaumberg, who notes that older people and obese persons tend to have higher levels of inflammatory activity in the body. "Obesity is one of the strongest contributors to the levels of something like C-reactive protein," she says.

Schaumberg also identifies heavy metal exposure as a risk factor needing more research. According to a study led by Schaumberg and published in the 8 December 2004 issue of *JAMA*, low-level lead exposure appears linked to cataract formation in men. Of 642 men ranging in age from 48 to 93 years, 122 were diagnosed with cataract. Bone scans determined that the men's long-term, low-level lead exposure was comparable to that of the general population. Men in the highest exposure group (8.17–35.0 micrograms per deciliter) had 2.5 times the risk of having cataract as the men in the lowest exposure group (1.0–3.0 micrograms per deciliter). "As far as we know, this paper . . . was really the only epidemiological study looking at heavy metals in relation to eye disease. I think it's really an area that we don't know much about," she says.

At the opposite end of the age spectrum, children may have cataract at birth or develop the condition in infancy due to prenatal infection with rubella or toxoplasmosis, among other causes. Such cases need immediate treatment. One consequence of untreated cataract is nystagmus, any of a variety of involuntary movements of the eyes. Amblyopia, or "lazy eye," is another condition found in children. "In the first weeks up until the first couple of months [of life], if vision is disturbed in both eyes that then will cause poor vision for the rest of life because nystagmus cannot be treated in any way," says Jill Keeffe, an associate professor at the Centre for Eye Research Australia. "With cataract, it needs to be treated within weeks, whereas with amblyopia, which might develop from strabismus [drifting or crossing of one or both eyes] or from uneven refractive error between the two eyes, the window of opportunity is much longer. Obviously, the earlier, the better."

Retinal Disorders

Retinal disorders also pose a threat worldwide. Age-related macular degeneration (AMD) refers to damage to the area of the retina responsible for sharp central vision. It is the third most common global cause of blindness, accounting for approximately 8.7% of total blindness, and the primary cause of blindness in developed countries. In the United States, there are approximately

All images: National Eye Institute

Insight into the problem. Flies attracted to eye secretions are one way trachoma is transmitted (above). In its bid to eradicate this disease by 2020, the WHO encourages facial cleanliness (right top) and improvements in sanitation such as burying waste (right bottom).

Clockwise from left: Elizabeth Gilbert/International Trachoma Initiative; Harvey Wang/International Trachoma Initiative; Elizabeth Gilbert/International Trachoma Initiative

1.8 million people with vision loss due to AMD, and another 7.3 million are at risk. As the average age of the world's population creeps upward, AMD will become even more significant.

As much as 30% of AMD may be related to smoking. A prospective study published in the 9 October 1996 issue of *JAMA* linked smoking with AMD, and more recently a study in the 14 April 2005 *British Journal of Ophthalmology* showed that smokers were twice as likely as nonsmokers to develop AMD. The risk declines if one stops smoking, to the point that after 20 years of not smoking former smokers have about the same level of risk as nonsmokers. Possible mechanisms of damage linked to smoking include depressed levels of antioxidants, reduced oxygen, and altered blood flow.

The effect of diet on AMD risk shares some of the same components as cataract; specifically, low-level antioxidant levels may heighten the chances of developing the disease. Obesity and high blood pressure, fat intake, and cholesterol levels also appear to increase AMD risk, but the specifics are not yet clear.

Family studies imply a genetic link, which is supported by three papers published in the 15 April 2005 issue of *Science* and a fourth published in the 2 May 2005 issue of *Proceedings of the National Academy of Sciences*. "Age-related macular degeneration is certainly a disease that's affected by our genes," says Timothy Stout, an associate professor of ophthalmology at the Casey Eye Institute in Portland, Oregon. "What's interesting is that it's also a disease that's influenced by our environment. The link between those two has been puzzling in the past, and I think there are new studies that suggest that some of the genes that play a role in the development of macular degeneration are genes that may be involved in inflammation and our immune response."

In early 2005, the four teams independently associated AMD with a gene coding for complement factor H, an inflammatory component. "The recent research implicating the complement factor H gene in AMD is a major breakthrough," says Peter Humphries, a professor of medical molecular genetics at Trinity College in Dublin, Ireland. "Many studies have resulted in localizing so-called susceptibility genes to chromosomal regions, but the studies recently reported are the first to identify an actual gene. I expect that we will find out a great deal more about the so-called molecular pathology of AMD as a result of this discovery."

Further, up to six regions within the genome have been implicated as potentially harboring AMD genes, and a second gene was reported in the November 2005 *Human Molecular Genetics*. "As yet we have very little information about this most recent gene," says Humphries. "[However], once more is known about the mechanisms of action of such variants, we stand to know a lot more about the cause of AMD, and hence the prospects for eventual prevention will become more realistic."

Retinal damage is also a hallmark of diabetic retinopathy, which blinds about 5 million people worldwide. The U.S. Centers for Disease Control and Prevention estimates that 13.8 million Americans have been diagnosed with diabetes and another 5.2 million have it without realizing it. Duration of diabetes and its control affect risk of diabetic retinopathy, and approximately 5.3 million Americans over age 18 have the eye condition. With diabetes rates increasing, in part due to increasing obesity, diabetic retinopathy can be expected to become more prevalent. Dietary and genetic factors may also affect its development, as may high blood pressure and high cholesterol.

"All of these things—hypertension, diabetes, hypercholesterolemia—tend to have

bad effects at the level of the small blood vessels, the capillaries," says Stout. Retinal vein blockages associated with high cholesterol, high triglycerides, and high blood pressure can create capillary-bursting pressure. The tissue then becomes hypoxic, or insufficiently oxygenated. Retinal hypoxia also occurs in diabetes when the capillary network dies through mechanisms that are not completely clear.

Hypoxia triggers production of vascular endothelial growth factor, which promotes formation of new blood vessels, but the process is disorganized. "The body tends to not build the blood vessels in the right place, so they will grow not as nicely formed capillaries in the retina, but at the optic nerve or into the center part of the eye or at the part of the eye where the drainage system is, and that causes all sorts of problems," says Stout. Further, the poorly built new vessels leak, as may existing blood vessels. Ultimately, the retina detaches from the underlying layers, and vision is lost.

Infections and Nutritional Deficiencies

Slightly more than 5% of global blindness arises from injury- or disease-associated corneal opacity. Distinct from this category, trachoma accounts for an additional 3.6% of global blindness. Trachoma is the most common infectious cause of vision loss and affects approximately 84 million people, primarily in remote rural areas of Africa, Asia, Central and South America, Australia, and the Middle East. This disorder arises from repeated infection by *Chlamydia trachomatis* bacteria that are spread by close contact with an infected person or by flies. After numerous infections, eyelid scarring turns the eyelashes inward, and they rake against the cornea, a condition called trichiasis. The irritation scars the cornea and eventually renders it opaque.

Infection typically starts in childhood, although the blinding effects do not occur until well into adulthood. "It's not just one infection—it's repeated infections," says Keeffe. "We've seen scarring in the lids of preschool-aged children, . . . but it's usually not until the forties and fifties that vision loss occurs." In some areas, 60–90% of preschool-age children carry active infections. Women account for 75% of late-stage blinding trachoma cases, possibly because they have greater contact with children.

In 1997 the WHO launched GET (Global Elimination of Trachoma) 2020 with the goal of eradicating trachoma altogether. A major part of GET 2020 is a primary health care plan known as the SAFE strategy. The SAFE strategy utilizes lid surgery (S), antibiotics (A) to treat active infections, facial cleanliness (F), and environmental changes (E) geared toward improving sanitation and access to clean water. A review of the SAFE strategy published in the June 2003 issue of *The Lancet Infectious Diseases* found strong support for the use of antibiotics and surgery in warding off infection and blindness, although the evidence for face washing and environmental improvements was weaker.

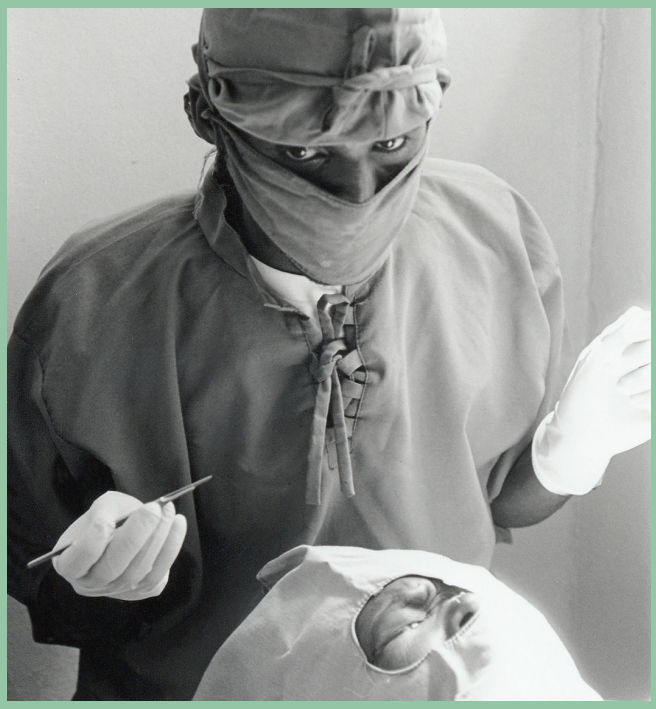
Onchocerciasis, also known as river blindness, is caused by *Onchocerca volvulus*, a parasite transmitted by blackflies in riverside areas. The disease is endemic in West and Central Africa, Yemen, and several South American countries. When a blackfly bites, a juvenile form of the parasite enters the body. Once mature, females release high numbers of larvae that migrate to the skin and eyes. Associated lesions form in all eye tissues except for the lens, and lead to inflammation, bleeding, secondary infections, and eventually blindness. More than 17 million people are infected with the parasite, approximately 500,000 people are visually impaired as a result, and another 250,000 are blind. Fear of infection prevents arable riverside land from being used, and local economic growth stagnates.

Global efforts at halting river blindness started with effective vector control efforts in 1974, and ongoing community-based treatment with ivermectin, an antiparasite medication, began in 1996. The disease has been reduced in most areas and may be eradicated from Latin America by 2010.

Among children, cornea-clouding vitamin A deficiency is the most common cause of preventable childhood blindness. Poor night vision is the key symptom of the very early stages of the corneal damage preceding blindness. At this stage, children can retain their vision with repeated doses of vitamin A. In late-stage vitamin A deficiency, the cornea becomes very white and cloudy, and vision loss is irreparable; cornea transplants are impossible because the tissue becomes too damaged. Immunization and good nutrition are key to preventing this form of blindness, but where these interventions are

Focusing on onchocerciasis. When parasites spread by blackflies (above) enter the body, their larvae migrate to the eyes and skin, causing lesions that result in inflammation, bleeding, and severe visual impairment or blindness if left untreated treated (right top). Treatment with ivermectin (right bottom) has reduced the disease in most areas and nearly eradicated it in some.

Clockwise from left: WHO/TDR; WHO/APOC/TDR/Crump; WHO/TDR/Crump



Seeing a way past trachoma. A 15-minute procedure performed by a doctor or trained nurse using local anesthetic can reverse the conditions that cause trachoma and reduce the risk of blindness. Globally, however, lack of access to basic health care keeps many patients in the dark.

not immediately possible, doses of vitamin A make "lovely primary health interventions," says Keeffe. In addition to supplying vitamin A supplements, health organizations also strongly promote vitamin A–rich diets. Breastfeeding provides ample vitamin A to babies, and older children and adults receive the nutrient through garden produce and supplemented foods.

An Eye to the Future

The WHO estimates that up to 75% of all blindness is preventable. Vision 2020: The Right to Sight, a program instituted in 1999 by the WHO and the International Agency for the Prevention of Blindness, builds upon previous programs, dovetails with pre-existing efforts of many organizations, identifies remaining regional and national needs, and provides a framework for filling gaps. Through Vision 2020, a coordinated effort is under way to eliminate preventable blindness by 2020 by increasing awareness of eye disease, garnering resources for prevention and treatment, controlling major causes of avoidable blindness, training ophthalmologists and other eye care personnel to diagnose and treat the diseases specific to certain regions, and providing these specialists with necessary technology and infrastructure.

Other organizations also have carried out large-scale international programs. For example, Lions Clubs International, a service organization with a long history of combating low vision and blindness, instituted the worldwide SightFirst program with three major goals: treating and preventing diseases such as river blindness, trachoma, and cataract; providing education and training of health care workers to diagnose and treat eye disease; and constructing and equipping health care facilities. The group is currently building SightFirst II as well as collaborating with the WHO on the Project for the Elimination of Avoidable Childhood Blindness.

On a national level, the U.S. government sponsors prevention and treatment through the Department of Health and Human Services' Healthy Vision 2010 and the NEI's National Eye Health Education Program. Healthy Vision 2010 is part of Health People 2010, a national program to improve the health of Americans, and seeks to promote regular eye examinations for adults and children, vision screening for preschoolers, and injury prevention. There's also a component to educate people with low vision about treatment. The National Eye Health Education Program has a more specific focus, encouraging early detection and treatment of glaucoma and diabetic eye disease, and providing education about treatment for low vision. This program provides materials that communities can use to educate the public about eye disease and the importance of early detection and treatment.

A critical gap in eliminating preventable blindness and low vision is delivery of health care. Without access to health care, opportunities are missed to diagnose problems early when treatment is most likely to be successful. Access to health care is a problem in many nations, including the United States. Stout offers diabetes as an example of the United States falling short in this regard. "That's a huge population problem for us in the United States, because as people are falling through the health care cracks and aren't [controlling diabetes] they're going to get very significant blinding diabetic retinopathy at a relatively early age," he says. "Vision loss in anybody is not a good thing, but vision loss in a relatively young, healthy person who presumably has a productive career ahead of them—it's a real issue."

For all that's been learned about eye disease, there remain gaps, and new hypotheses continue to be generated. Researchers at the Schepens Eye Research Institute recently embarked on the $2.2 million, three-year Planning Grant for Research on Blinding Eye Diseases. Like other NIH "roadmap" grants, this one is designed to promote interdisciplinary collaborations regarding complex health challenges. "While experts from these areas often collaborate informally on [eye] disease, ophthalmology has remained somewhat specialized and in some ways isolated from other disciplines," remarked Darlene Dartt, director of scientific affairs at Schepens Eye Research Institute, in announcing the grant. "This is really the first federal program to formalize collaboration." In learning more about the cascade of events occurring in other parts of the body in diseases ranging from Alzheimer disease to rheumatoid arthritis, researchers might gain some insight into the processes of blinding eye diseases.

Julia R. Barrett

All images: Elizabeth Gilbert/International Trachoma Initiative

GAO Sounds Off on Chemical Regulation

Hazlan Abdul Hakim/iStockphoto

Since 1976 the Toxic Substances Control Act (TSCA) has given the federal government the power to require that chemicals are properly tested and regulated before they reach the market, and that they don't pose unreasonable risks to human and environmental health. TSCA is the key piece of legislation governing the way the U.S. Environmental Protection Agency (EPA) reviews and regulates chemicals including solvents and constituents of paints, fuels, and plastics. Yet, concerns persist about chemical safety and the adequacy of regulation.

Now, in a June 2005 report titled *Chemical Regulation: Options Exist to Improve EPA's Ability to Assess Health Risks and Manage Its Chemical Review Program,* the Government Accountability Office (GAO) has reviewed the EPA's efforts to control the risks of new chemicals not yet in commerce, to assess the risks of existing chemicals used in commerce, and to publicly disclose information provided by chemical companies under TSCA. The report points out shortcomings in TSCA and its implementation, and suggests ways to strengthen the law.

HPV Chemicals

TSCA authorized the EPA to both assess new chemicals before they enter the marketplace and to review chemicals already on the market. But when the law was enacted, thousands of chemicals already being used were grandfathered in. "Those sixty-two thousand or so chemicals were just accepted as being okay to be in commerce without any kind of EPA risk analysis," says David Bennett, the report's lead analyst.

Even aside from these grandfathered chemicals, the EPA has an enormous number of chemicals to examine, so the agency has narrowed its approach. "We have decided to focus our work on the high-volume chemicals, using volume as a surrogate for [human and environmental] exposure," says Charles Auer, director of the EPA Office of Pollution Prevention and Toxics. The High Production Volume (HPV) Challenge Program was started in 1998 with the goal of looking at some 2,800 chemicals that were produced in quantities exceeding 1 million pounds per year as of 1990. The voluntary program was established by the EPA, Environmental Defense, the American Chemistry Council (ACC), and the American Petroleum Institute to identify and fill gaps in basic hazard data for these chemicals, and to make those data publicly available by 2005.

The information garnered from the HPV Challenge Program "will allow us to prioritize among the chemicals and then obtain additional information where appropriate or take control actions," says Auer. Michael P. Walls, managing director of health, products, and science policy at the ACC, adds, "Through the [HPV Challenge] Program we provide a mechanism to assure the agency that there is adequate information on which to base current risk management decisions."

"The program is a light-some-candles-rather-than-sit-and-curse-the-darkness initiative," says Karen Florini, a senior attorney with Environmental Defense. "It gathers preliminary basic screening-level information. It's clearly valuable; it's just limited."

Despite progress made to date, the GAO report states there are 300 chemicals in the HPV Challenge Program "for which chemical companies have not agreed to provide the minimal test data that EPA believes are needed to initially assess

their risk." Auer regards that situation as "unfinished business." He says the EPA is developing rules to require industry to test those chemicals. "We hope to finalize that test rule at the end of this year or early next year," he says.

Both Auer and Walls note there could be a variety of reasons for the chemical industry not doing this testing voluntarily. For example, in some instances domestic manufacturers of a chemical have said they would be willing to provide information only if foreign competitors who export the chemical to the United States would share the cost of testing—support that was not forthcoming. However, once a test rule is in effect, anyone who produces or imports the chemical must comply and provide the data.

New Chemicals

The GAO report also voices concern about the EPA's efforts to regulate new chemicals. Though the report noted that the EPA has taken actions to regulate exposure to about 3,500 of 32,000 new chemicals submitted for review since TSCA was enacted, the GAO has qualms about the way in which those chemicals were examined.

The EPA typically does not have enough data on a submitted chemical's properties to determine its toxicity. Consequently, it may compare a new chemical with closely related model chemicals to predict whether the new compound will pose a safety hazard. "We found evidence that in some cases the models were not entirely predictive. The problem is that in some cases there is just not a lot of data out there to show how predictive the models are," says Bennett.

Auer counters that the models do what they are supposed to—identify potentially hazardous candidates for further testing. He adds that the models also tend to err on the side of caution—that is, they tend to identify chemicals that appear to be hazardous but prove to be safe upon further examination.

The GAO report also notes that TSCA does not require chemical companies to submit data to the EPA on the toxicity, routes of exposure, or potential extent of exposure of new chemicals. "I think it is scandalous that new chemicals can be brought to market without being accompanied by any actual data," Florini says. "Eighty-five percent of PMNs [premanufacture notices, which must be submitted to the EPA at least 90 days before production of a new chemical begins] are submitted with no health data, and reliable models aren't available for many end points, particularly for long-term health effects other than cancer."

But Walls says chemical manufacturers must be prepared to supply data to the EPA if a chemical has characteristics of persistence, bioaccumulation, and toxicity. "The assumption that new chemical applications are filed in the United States with no information is not right," he says. He agrees that TSCA does not require safety and exposure data, but adds that if a company is submitting a chemical to which people could be exposed, the company would be "remiss" in not providing that information. Still, the data need be provided only if the agency asks for them.

Auer asserts that requiring toxicity testing before a chemical is actually manufactured, as required by TSCA, could interfere with innovation in the industry. He estimates the cost of providing the information wanted by the EPA to be in the range of a quarter of a million dollars for new chemicals. Auer says the expense of testing before a chemical company knows whether there will be a demand for a chemical could hobble efforts to develop improved chemicals, and maintains that the EPA's track record of taking action to reduce the risk of new chemicals is a good one.

TSCA, Take Two

Faced with the lack of required data for new chemicals and what the GAO regards as the uncertain effectiveness of the voluntary HPV Challenge Program, the report recommends that Congress give the EPA the authority to require chemical manufacturers to generate and provide test data on HPV chemicals. That recommendation is embodied in legislation introduced in Congress this summer by senators Frank Lautenberg (D–NJ) and James Jeffords (I–VT). The bill also would give the EPA the authority to require those data for all chemicals used, and to prioritize which chemicals the industry would have to test. Auer says the EPA has not yet taken a position on the bill. Environmental Defense supports it, while the ACC opposes it, saying it duplicates the EPA's existing authority under TSCA.

The GAO offers a number of other recommendations to strengthen the act. Among them are validating and improving the models used by the EPA to assess risks of chemicals; requiring chemical companies to submit testing results of chemicals with PMNs; letting the EPA regulate chemicals if they pose a "significant" risk to health or the environment rather than the more stringent "unreasonable" risk; and setting national goals for reducing the overall use of toxic chemicals.

Auer says the EPA will soon have much better exposure information under amendments to the TSCA Inventory Update Rule, which requires chemical manufacturers to submit basic production data every four years for chemical substances (including imports) manufactured for commercial purposes in amounts of 25,000 pounds or more at a single site. According to the EPA, the 2003 amendments tailor reporting requirements to more closely match the EPA's information needs, provide a vehicle for the EPA to obtain updated information on the potential human and environmental exposures of chemical substances listed on the TSCA Inventory, and improve the utility of the information reported under the rule.

With the amendments, "we will know the chemicals that are in consumer products," Auer says. "We will know the number of workers that are exposed to chemicals. We will have a better basic idea of the uses of chemicals. So EPA in sixteen months will have basic exposure information on [HPV] chemicals."

Moreover, Auer says the EPA will require the information to be updated every five years so the agency can understand how chemical use is shifting and whether safer alternatives have been developed in the meantime. He says the information will allow the EPA to identify HPV chemicals that are candidates for more testing or for enhanced regulation. Further, Walls says the chemical industry on its own initiative will supply the EPA with hazard data on around 500 chemicals that reached the high-volume threshold between 1990 and 2002.

Nevertheless, Bennett reserves judgment on the HPV Challenge Program's effectiveness. "The process is ongoing, so I would hesitate to say how successful it is, because EPA has not received all the data that industry has promised to deliver. We won't know for some time whether the program will be successful," he says.

Florini echoes that view, and also points out that the EPA is "way behind" in making public the information submitted to date. "Six years into the HPV Challenge Program, it's really regrettable that the database hasn't yet been released, though it apparently will be out by year-end," she says.

After three decades of existence, it is appropriate that TSCA is undergoing significant examination, as the EPA, the chemical industry, environmentalists, and legislators all look at ways to revise this major statute. What a revised TSCA will look like after this examination, however, is far from certain.

Harvey Black

Photodisc, Chris Reuther/EHP

Making Succinate More Successful

What does the word "fermentation" bring to mind? Beer? Bread? Ethanol derived from corn and other plant matter? How about succinate? Since 2001, biochemist George Bennett and bioengineer Ka-Yiu San, both professors at Rice University, have been tinkering with *Escherichia coli* to coax it to convert sugars to succinate, a chemical with multiple industrial uses. Now their efforts are bearing fruit as "green" succinate is starting to become a reality in chemical commerce.

Who uses succinate? By itself, succinate is used as a flavor enhancer in food products and as a stabilizer in pharmaceuticals. It is also used to produce other industrial chemicals, including butanediol, tetrahydrofuran, and pyrrolidone, which become ingredients in solvents, paints, deicers, plastics, fuel additives, fabrics, and carpets.

Succinate is traditionally manufactured from petrochemicals through expensive processes. The Rice team's goal is to make a more environmentally friendly succinate from renewable starting materials. "We want to use agricultural materials that are renewable to make this useful product, and alleviate the drain of limited oil reserves," says Bennett.

The Department of Energy (DOE) "sees a future for biorefineries that use biomass as feedstocks to make fuels and chemicals," says department chemist Gene Petersen. In 1994, the agency's now-defunct Alternative Feedstocks Program assessed the likelihood of making chemicals from biomass. "The category of compounds that seemed most viable were organic acids like succinic, acetic, and citric," says Petersen.

That evaluation resulted in the DOE's funding of fermentation research programs at national laboratories and universities. In 2004, the DOE released volume I of a report titled *Top Value Added Chemicals from Biomass*, coauthored by Petersen (volume II is expected out in 2006). According to the report, succinate tops the list of 12 "building block" chemicals—molecules with multiple functional groups that possess the potential to be transformed into new families of useful

materials—that can be produced from sugars via biological conversion.

In 2001, 10 million pounds of succinate were produced from petrochemicals and sold for an average of $2 per pound. "The market is there if we can make succinate more economically through biofermentation," says Praveen Vadlani, principal research scientist at AgRenew Incorporated in Manhattan, Kansas. By making green succinate in bulk—a potentially cheaper material with the cachet of environmental friendliness—people may even be inspired to find new applications for it, such as bio-based polymers and composites, predicts Vadlani.

Optimizing Glucose

"It's not a direct route from glucose to succinate," says Bennett. Several biochemical pathways can produce succinate from sugar. They all start with the degradation of glucose, which contains six carbon atoms, to pyruvate, which contains three carbons. Then pyruvate can be converted not only into succinate (which contains four carbons), but also lactate, ethanol, acetate, and other chemicals. The trick is to speed up the chemical reactions that lead to succinate production while blocking those that make lactate, ethanol, and other chemicals.

Some pathways operate aerobically (they need oxygen) whereas others run anaerobically (they do not use oxygen). Bennett and San spent four years working out both aerobic and anaerobic methods for *E. coli* to convert glucose into almost pure succinate in yields high enough to be commercially feasible. Their anaerobic method has proven more efficient, with 1.0 gram of glucose yielding 1.44 grams of sodium succinate. Their aerobic process yields about three-quarters that amount.

Bennett and San have engineered a form of *E. coli*, dubbed SBS550MG, that contains six genetic alterations that allow it to produce succinate anaerobically from two different routes—the glyoxylate pathway and the fermentation route. To accomplish this, the researchers deleted four *E. coli* genes, including those for lactate and ethanol production, and activated the glyoxylate pathway in order to speed the conversion of glucose solely into succinate. They also added two genes from other bacteria to boost the amount of succinate generated.

Both routes produce succinate through different biochemical reactions that do not compete or interfere with each another. In fact, Bennett and San designed the routes to be complementary. SBS550MG converts glucose to succinate very efficiently and very rapidly, and gives high yields of nearly pure succinate with few by-products, says San. High-pressure liquid chromatography confirms that more than 90% of the starting glucose ends up as succinate.

To make the leap from the laboratory to the marketplace, the Rice scientists teamed up with bioengineering experts at AgRenew. Under Vadlani's direction, AgRenew will perfect the methods to manufacture succinate from corn and sorghum rather than from the pure glucose used in the laboratory experiments. "We see great promise in the technology, and once the methods are established, we may even switch to cornstalks or agricultural waste," says Vadlani.

Up and Running

Kris Berglund, chief science officer at Diversified Natural Products (DNP) in Scottville, Michigan, is experiencing new market demands for green succinate. DNP

Sweetening the deal. Researchers are refining techniques for producing succinate from biomass such as sorghum (above) rather than petroleum. One group, MBI International, uses ion exchange to further refine succinate into succinic acid (right).

Left to right: Photodisc; MBI International

also uses *E. coli* to ferment sugars to succinate, but the bacterial strain used was licensed from the DOE, which produced it under its Alternative Feedstocks Program. DNP's fermentation method differs from that created by Bennett and San in that an aerobic process occurs first, followed by an anaerobic process that requires added carbon dioxide. Says Berglund, "We take six carbons from glucose and add two carbons from carbon dioxide to form two molecules of succinate with four carbons each."

DNP just started large-scale production of succinate from agricultural materials at Agro-Industrie Recherches et Développements (ARD) in Pomacle, France. The joint venture was announced by French president Jacques Chirac on 30 August 2005. In seeking a partner to manufacture its biosuccinate batches, Berglund searched worldwide and chose ARD because "they shared the same vision as we do to replace petroleum-based chemicals with biomass production," he says.

The staff at ARD's manufacturing facility, located in the agricultural Champagne region, will produce up to 200 tons of succinate from wheat and sugar beets in the first year. DNP plans to construct a large plant in the United States, too. "As far as we know, we're the first company to enter commercial production of succinate from biomaterials," says Berglund. Although production has just begun, Berglund says "customers already want to buy it," particularly for use as a flavor enhancer, stabilizer, and acidulant for food production. Some customers desire green succinate because they view it as a "natural" ingredient that would be favored by organic food consumers.

Customers also are lining up to buy DNP's succinate-based runway and wing deicer. Succinate, which lowers the freezing point of water, replaces the formates and acetates in deicers now on the market. These chemicals not only corrode the metal alloy, plastic, and rubber parts of airplanes, but also destroy the concrete surfaces and plastic and metal components of lighting equipment at airports. Federal Aviation Administration approval of the DNP deicer appears imminent, according to Berglund. Other products in the succinate pipeline at DNP include biodegradable solvents that do not cause air pollution or damage the ozone, a diesel fuel additive to reduce particulate emissions, and biodegradable polyesters for use in fabrics or plastics.

DNP does not disclose information about its yields, but "our methods are good enough to compete with any fossil fuel–related process," says Berglund. Based on estimates calculated when oil sold at $25 per barrel, DNP forecast a selling price of less than $1 a pound for its biosuccinate. With declining petroleum reserves and rising oil prices, "the economics of our process are even more attractive," says Berglund.

Other companies are following behind on the same commercialization path. Michigan Biotechnology Institute (MBI) International in Lansing developed a patented process based on *Actinobacillus succinogenes*, a bacterium isolated from the cow's rumen (a fermentation chamber in the animal's stomach). MBI scientists created mutant strains for anaerobic production of succinate from biomass sugars, resulting in yields of approximately 1 gram of succinate from 1 gram of glucose. Different types of biomass, including cornstalks, corn fiber, and sugarcane, can be used to fuel the fermentation.

The MBI method also pipes in carbon dioxide. "It's a greenhouse-friendly fermentation, because we utilize carbon dioxide instead of generating carbon dioxide," says microbiologist Bernie Steele, manager of quality assurance at MBI. He foresees his company's biosuccinate method being linked to ethanol plants, which generate carbon dioxide as a waste product. An overall biorefinery program that uses by-products from one production stream to feed another manufacturing process maximizes economic returns.

After 10 years of research and development efforts, MBI is seeking partners to scale up its process to manufacture large quantities of green succinate. "The technology is maturing for the transition of biomass into energy or chemicals," says Steele.

Future Uses for Succinate

The future for succinate lies not in utilizing it directly as a food additive, but in creating innovative biopolymers like polybutylene succinate. This biodegradable plastic, already being made with petroleum-based succinate, is found in packaging film, bags, flushable hygienic products, and garden mulch. "We have customers waiting to buy our succinate to make polymers," says Berglund. Other, stiffer biodegradable plastics, like polylactic acid, are formed into drinking cups, food trays, containers, and planter boxes. These "green" alternatives replace products typically made from petroleum-based plastics.

The commitment of corporate giants like Cargill and DuPont to make products from biomass casts "a bright light on the future of biofermentation," says Petersen. Cargill produces up to 300 million pounds of polylactic acid, sold as NatureWorks® PLA, from renewable resources such as corn. DuPont's Sorona®, a polymer of 1,3-propanediol now made from petrochemicals, adds softness and stretch to fabrics. In 2006, DuPont will switch to a fermentation method to make its 1,3-propanediol from corn sugar. Called Bio-PDO™, the corn-based polymer will be the first product developed by DuPont's Bio-Based Materials unit.

Despite this buy-in, the future isn't here yet. In general, the long journey to find an economic way to convert renewable biomaterials into commodity chemicals takes about 10 years; the basic research behind NatureWorks PLA started in the 1980s. "It's not that easy to get away from petrochemicals, even though we want to environmentally," says Petersen.

But the large-scale processes under way at Cargill and DuPont indicate long-term business interest in fermentation, says Bennett. He envisions more companies entering the bioproducts business and the economics of succinate and other bioproducts improving through engineering refinements. And as oil prices rise and fermentation becomes more economically appealing, "companies will find different ways to make the same end product," says Vadlani.

Carol Potera

Suggested Reading

Chatterjee R, Millard CS, Champion K, Clark DP, Donnelly MI. 2001. Mutation of the *pstG* gene results in increased production of succinate in fermentation of glucose by *Escherichia coli*. Appl Environ Microbiol 67(1):148–154.

Guettler MV, Rumler D, Jain MK.1999. *Actinobacillus succinogenes* sp. nov., a novel succinic-acid-producing strain from the bovine rumen. Int J Syst Bacteriol 49 (pt 1):207–216.

Sanchez AM, Bennett GN, San KY. 2005. Novel pathway engineering design of the anaerobic central metabolic pathway in *Escherichia coli* to increase succinate yield and productivity. Metab Eng 7(3):229–239.

Werpy T, Petersen G, eds. 2004. Top Value Added Chemicals from Biomass. Volume I—Results of Screening for Potential Candidates from Sugars and Synthesis Gas. Springfield, VA: National Technical Information Service. Available: http://www.nrel.gov/docs/fy04osti/35523.pdf.

Asthma in Young Children

Prenatal DDE Exposure May Increase Risk

Most countries have banned the agricultural use of the organochlorine insecticide DDT because of the way this persistent, fat-soluble compound accumulates in the food chain. However, DDT is still widely sprayed in developing countries to combat malaria-bearing mosquitoes. Studies have linked exposure to DDT and its persistent metabolite *p,p′*-DDE to changes in the immune responses of human cells, and to asthma prevalence in children and adults. A longitudinal study now shows that prenatal exposure may provide the fundamental window for asthma susceptibility linked to DDT [***EHP* 113:1787–1790**].

Investigators collected umbilical cord blood from 482 children born on the Spanish island of Menorca and tested 84% for the presence of organochlorine compounds. DDT is not used on Menorca. However, the parents of the children in the study ate relatively large amounts of fish, which can be a source of exposure to DDT residues. According to self-reports of diet on questionnaires, more than half of the mothers ate fish more than twice a week during pregnancy.

All of the children tested had *p,p′*-DDE in their cord serum (the median concentration was 1.03 nanograms per milliliter [ng/mL]). Serum levels tended to be higher in children with older mothers. All of the children's serum also contained hexachlorobenzene and polychlorinated biphenyls.

Then and now. A study of Spanish mother–child pairs shows that DDT exposure *in utero* may contribute to later asthma in children.

The researchers correlated the children's prenatal exposure to risk of having asthma or atopy at age 4. Asthma was defined as one or more episodes of wheezing in the fourth year alone, one or more episodes of wheezing per year in consecutive years ("persistent wheezing"), or a physician's diagnosis of asthma. Atopy was defined as having blood levels of specific immunoglobulin E antibodies for dust mites, cats, or grasses. Of the initial participants, 97% provided medical information yearly through age 4 and 75% provided blood samples at age 4; 306 of these samples were tested for antibodies and for peripheral white blood cells, a sign of the underlying inflammation responsible for asthma.

Wheezing was reported at age 4 for 11.6% of the children whose blood was tested for organochlorines. In addition, 12.6% of those who gave blood at age 4 had antibodies for the specified allergens in their blood. The risk of wheezing increased with the concentration of *p,p′*-DDE in the child's cord serum. Of the children in the lowest quartile of exposure (less than 0.57 ng/mL), 9% reported wheezing compared to 19% of the children in the highest quartile of exposure (more than 1.90 ng/mL). There were no correlations between wheezing in the children and maternal consumption of fish during pregnancy.

There was no apparent link between atopy and the relationship between DDT and wheezing; children both with and without atopy had a similar increase of wheeze with increasing *p,p′*-DDE. The researchers speculate that the lack of an association between DDT exposure and atopy in their study could be due to the young age of the children studied, as sensitization to allergens tends to increase during childhood. There was no correlation between the other organochlorine compounds measured and wheezing or atopy.

Further study is needed to determine if the link between DDT and asthma susceptibility is caused by the effect of the insecticide on the immune system or the hormonal system. In addition to its direct impact on immune cells as shown in previous research, *p,p′*-DDE has also been shown to interfere with hormonal receptors and to mimic estrogen activity, which might indirectly affect immune responses. The researchers suggest that their results be considered when evaluating the risk of spraying DDT in antimalaria campaigns. **–Kris Freeman**

Death by Particles

The Link Between Air Pollution and Fatal Coronary Heart Disease in Women

A growing body of evidence links chronic exposure to air pollution—especially particulate matter (PM)—with mortality resulting from a variety of heart, lung, and respiratory diseases. A new study corroborates this association, and indicates that women may be at greater risk than men of fatal coronary heart disease (CHD) as a result of exposure to airborne PM [***EHP* 113:1723–1729**]. When ozone (O_3) or sulfur dioxide (SO_2) is also present, women's risk appears even greater.

The study, by a team of epidemiologists at Loma Linda University, is part of the 22-year Adventist Health Study on the Health Effects of Smog. It followed 3,239 nonsmoking, non-Hispanic white adults in several mainly urban areas in California from 1976 to 1998. The researchers associated CHD deaths with prior exposure to various levels of several common

Digital Stock

air pollutants: $PM_{2.5}$, $PM_{10–2.5}$, PM_{10}, O_3, SO_2, and nitrogen dioxide (NO_2).

Participants completed a baseline health and lifestyle questionnaire in 1976, and four subsequent questionnaires covering personal sources of air pollution, such as secondhand tobacco smoke and fumes in the workplace. The researchers used airport visibility measurements (for $PM_{2.5}$ only) and data from state-run air pollution monitors (for all other pollutants) to estimate pollutant levels over time for the zip code centroids of participants' work sites and residences. Documented pollutant levels ranged from negligible to above legal limits. California's death certificate files and the National Death Index provided data on numbers and causes of deaths.

The researchers found that CHD caused 23.7% of all the deaths in the study cohort (155 women and 95 men). Adjusting for past smoking, body mass index, education level, frequency of eating meat, and calendar year (as PM levels declined over the study period), the researchers conducted statistical analyses to determine whether fatal CHD was associated with long-term exposure to the pollutants, either singly or in combinations of single gases and PM.

Women showed a relative risk for fatal CHD of 1.42, 1.38, and 1.22 with each increase of 10 micrograms per cubic meter ($\mu g/m^3$) of airborne $PM_{2.5}$, $PM_{10–2.5}$, and PM_{10}, respectively, in the air pollution they encountered during the four years preceding death. Postmenopausal women showed higher relative risks of 1.49, 1.61, and 1.30 for each 10 $\mu g/m^3$ increase in $PM_{2.5}$, $PM_{10–2.5}$, and PM_{10}, respectively. Neither O_3, SO_2, nor NO_2 was associated with fatal CHD on its own. O_3 and to a lesser degree SO_2 (but not NO_2) increased the effect of all sizes of PM. O_3 in conjunction with $PM_{2.5}$ yielded the most striking results: a relative risk of 2.0 in all women. Contrary to findings from several other studies that found increased risk of cardiopulmonary deaths due to PM in both genders, men showed no response to any of the pollutants.

The researchers highlight several physiological mechanisms that may explain their findings. Short-term exposure to PM is known to increase arrhythmia, inflammation, and blood viscosity, and to decrease heart rate variability, among other adverse effects that could lead to fatal CHD. Other findings show that O_3 exposure increases lung permeability, perhaps easing PM's entry into the bloodstream. Finally, several studies have indicated that PM deposits differently—and perhaps more harmfully—in women's lungs than in men's. This may provide a starting point for teasing out the study's finding of an association between PM and risk of fatal CHD in women, but not in men. **–Rebecca Kessler**

Tiny killer. Chronic low-level exposure to aflatoxins produced by *Aspergillus* molds (such as *A. flavus*, above) is associated with increased risk of liver cancer.

Liver Cancer and Aflatoxin

New Information from the Kenyan Outbreak

Millions of people are exposed to aflatoxins, toxic compounds produced by *Aspergillus* molds. These molds infest staple crops such as maize, peanuts, rice, and wheat throughout the world. Outbreaks of aflatoxicosis affecting up to several hundred people at a time have occurred sporadically, most recently in eastern Kenya in early 2004. An investigation of the Kenyan outbreak now yields new information on the risk factors associated with acute aflatoxin poisoning [***EHP* 113:1779–1783**].

Chronic low-level exposure to aflatoxins, particularly aflatoxin B_1, is associated with increased risk of developing liver cancer, impaired immune function, and malnutrition. Acute high-level exposure, which is less common, causes early symptoms of diminished appetite, malaise, and low fever. Later symptoms, including vomiting, abdominal pain, and hepatitis, signal potentially fatal liver failure.

The Kenyan outbreak followed a poor harvest of maize that had been damaged and made susceptible to mold by drought. Furthermore, to guard against theft of the meager harvest, people stored the maize in their homes, which were warmer and moister than the granaries where it was usually stored. From January to June 2004, 317 people sought hospital treatment for symptoms of liver failure, and 125 died. Health officials ruled out viral liver diseases; suspecting acute aflatoxin poisoning, they examined maize samples and found aflatoxin B_1 concentrations as high as 4,400 parts per billion (ppb), 220 times the Kenyan limit for food.

Researchers conducted a case–control study using records for 40 patients (cases) who had been hospitalized with acute jaundice during late May and early June and 80 randomly selected controls. Jaundice is a nonspecific symptom of liver damage.

Dennis Kunkel Microscopy

Participants or family members completed questionnaires targeting maize quality, storage, preparation, and consumption. The researchers collected 1-kilogram samples of maize from households that still had grain left over from the time of the outbreak for measurement of aflatoxin concentrations. Blood samples from 29 patients and 62 controls were analyzed for concentrations of aflatoxin B_1–lysine albumin adduct, a marker of aflatoxin exposure. The researchers also tested blood from 18 patients and 54 controls for hepatitis B surface antigen, an indicator of hepatitis B infection. In people with chronic low-level aflatoxin exposure, this virus enhances the risk of developing liver cancer.

Maize from patients' homes contained significantly higher amounts of aflatoxin (with a geometric mean of 354.5 ppb) compared to control households (with a geometric mean of 44.1 ppb). Patients' serum aflatoxin adduct concentrations, which were comparable to those measured in previous outbreaks, were nearly 10 times higher than those of controls. Further, patients who died had higher blood levels of adducts than those who survived. Forty-four percent of the patients tested positive for hepatitis B, compared to 7% of controls.

These analyses, with their greater level of detail, are the first to quantify the association between concentrations of aflatoxin in food, exposure history, concentrations of serum aflatoxin adducts, and acute aflatoxin poisoning. This study is also the first to quantify the independent association between hepatitis B infection and the effects of acute aflatoxin poisoning. The researchers suggest that monitoring both aflatoxin concentrations in crops and the incidence of acute jaundice could permit earlier recognition of food contamination and help prevent an outbreak from becoming widespread. Further, they suggest that future use of blood tests for aflatoxin B_1–lysine albumin adducts could serve to diagnose aflatoxin poisoning and to gauge the success of measures for reducing aflatoxin exposure. **–Julia R. Barrett**

Double jeopardy. Ergonomic stress may heighten the threat posed by on-the-job lead exposure.

The Heavy Load of Lead

Ergonomic Stress Heightens Exposure-Related Neuropathy

Long-term lead exposure among industrial workers can result in neuropathy (a disorder of the peripheral nervous system), while lower exposure levels cause muscle weakness. Until recently, however, the interaction between lead toxicity and chronic repetitive muscle use had not been investigated. Researchers from the Center for Occupational and Environmental Neurology in Baltimore now report that the impact of chronic lead exposure is augmented by concomitant ergonomic stress [***EHP* 113:1730–1734**].

The study included 80 lead smelter workers who were routinely exposed on the job to inorganic lead dust and (to a lesser extent) lead fumes. Historical blood lead records for all the workers were available from the smelter, which checked all employees' blood lead at least quarterly. These records showed that workers had high chronic exposure in the distant past, much lower exposure in the more proximate past, and still lower exposure at the time of the study. The researchers also measured current blood and bone lead levels and used the historical records to calculate two metrics of cumulative lead exposure—working-lifetime integrated blood lead (IBL) and working-lifetime weighted-average blood lead (TWA).

The team used the current perception threshold test to examine nerve fiber populations in the workers' shoulders, arms, wrists, and hands. This test measures the amount of electrical current needed to induce a sensation. The team also created a three-tiered ergonomic stress rating based on all the different jobs the workers had ever performed, cumulated over their employment history. This was used to arrive at a time-weighted average ergonomic stressor. Sensory nerve conduction threshold was measured in large myelinated, small myelinated, and unmyelinated nerve fibers.

The results showed that decrements in nerve function—a precursor to neuropathy—were limited to large and small myelinated sensory nerve fibers, with a threshold effect at a TWA of 28 micrograms per deciliter. At higher levels of lead exposure and presence of ergonomic stress, nerve fibers were more susceptible to increased damage, something that has never before been shown in human studies. The investigators suggest that nerves affected by lead are more susceptible to traction or mechanical compression, as would occur in the carpal tunnel of workers who perform activities such as heavy lifting and shoveling.

Measures of chronic lead exposure may serve as strong predictors of impaired nerve function. In addition, the authors believe they have been able to separate the impact of two components of cumulative blood lead—duration and intensity—with exposure intensity appearing to have a greater influence than duration on the outcome studied. Finally, the authors point out that although TWA and IBL are associated with peripheral nerve damage, bone lead—another measure of chronic exposure—is a weak predictor of lead effects in the nervous system because it reflects only that lead stored in the bone compartment and not necessarily the cumulative blood lead to which peripheral nerves were exposed. **–Dinesh C. Sharma**