Bright Ideas

"With a coronavirus like SARS, *there's a lot of information in place that can be harnessed to develop treatments."* —MARK DENISON

Old Virus, New Tricks

In the last 30 years, scientist Mark Denison estimates, researchers have been identifying something on the order of one new humanly recognized or newly emerging human infectious disease per year. Advances in the science of microbiology are part of the reason. But Denison says the rate of identification also reflects the fact that human beings make it so easy for viruses to thrive.

There are 6.3 billion of us dispersed over all parts of the planet, often living in close quarters or jetting across continents. And when we're not spreading viruses around among ourselves, we're relocating other species or planting monocrops that make it easy for viruses to spread—as in the case of the Irish potato famine.

Denison, associate professor of pediatrics and associate professor of microbiology and immunology, is an expert in coronaviruses, which are responsible for, among other things, 30 percent of common colds. For the past 11 years, Denison has quietly gone about his work in the Lamb Center for Pediatric Research, studying the mouse hepatitis virus to increase our understanding of how viruses grow and spread.

Since March, however, the work of the Denison lab has been under an intense spotlight. The SARS (severe acute respiratory syndrome) virus, which has devastated parts of Asia and created a worldwide scare, is a coronavirus. Suddenly, everyone is coming to Denison for information. He is collaborating with the National Institutes for Health (NIH) and the Centers for Disease Control (CDC) to understand the replication process of the SARS virus.

Speaking to *Vanderbilt Magazine* on a recent day during which he had also given interviews with CNN, *Newsweek*, and a Nashville television station, Denison says that, as ominous as the threat from SARS may be, medical researchers are far ahead of where they were when HIV first appeared two decades ago.

"When HIV first appeared, we didn't know the virus. We didn't know how it grew, or what kind of cells it grows in, or anything about the enzymes or proteins." However, with a coronavirus like SARS, Denison says, "there's a lot of information in place that can be harnessed to develop treatments or prevention strategies.

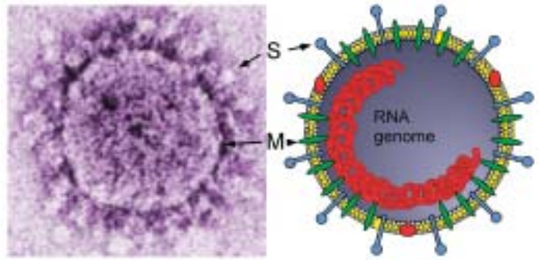
"Coronaviruses live out on the edge of the RNA world," he adds. "RNAs usually can't be very big, but the coronavirus RNA is huge." The range of diseases they can cause in animals includes severe respiratory disease, hepatitis, neurological disease, and renal disease. They have also been used as an animal model for studying multiple sclerosis.

Coronaviruses are found in many animal species. "They have some fairly novel characteristics," Denison says. "I call them promiscuous viruses. They do everything big. Their genetic material is big, they make a lot more proteins than other viruses, and they mix and match their genetic material during their normal life cycle. So they can generate lots of different variances."

A virus that could live for thousands of years in one host animal species, for example, might suddenly mutate or mingle with another virus, allowing it to jump to another type of animal.

Denison speculates that such a variance could have allowed SARS to jump from another as-yet unknown animal species to humans. "Any time something new is introduced into a genetically and immunologically naïve population, it's risky," he says.

Scientists are looking at more than one approach to attacking the SARS virus. The one in which Denison and his colleagues are most interested involves preventing the virus from making proteins. When the virus enters the host cell, it makes a polyprotein, a giant protein that contains a number of substances, including three enzymes called proteinases. The proteinases chop up the polyprotein into smaller, functional proteins.



Difficult to grow under laboratory conditions, coronaviruses are responsible for SARS and 30 percent of common colds.

Dr. Mark Denison

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"We've shown that if you can block this process, the virus can't go on," Denison says. "So we're particularly interested in proteinase inhibitors—enzymes or chemicals that keep the virus from cutting its own replication proteins."

Proteinase inhibitors are used in the treatment of HIV. New ones would likely need to be developed to treat SARS.

"We're also interested in working with the NIH and the CDC to develop a SARS vaccine," Denison says. He is particularly interested in live attenuated vaccines, which have a reduced capacity to grow—as in the case of the oral polio vaccine. "They can still grow and infect people, but because they grow poorly, the body has time to build up an immune response."

Denison's research with the mouse hepatitis virus has already shown that, by producing a mutation of that virus, it is possible to stop the virus from cutting the polyprotein into smaller individual proteins, making it harder for the virus to grow.

"I've worked with coronaviruses for 18 years, and some of my colleagues have worked with them even longer. We know an awful lot," he notes. "SARS is like the kid down the street. We know everything about him. We know his actions and movements. We just didn't expect him to commit a crime. But we have a lot of places to start. And that's a compelling argument for the value of doing basic science. The research we've done over a period of years is now paying huge dividends because with SARS, it's allowed us to learn so much very quickly."

Personal Theories and the Diagnosis of Mental Disorders

2. When your therapist tells you you're depressed or bipolar or have a borderline personality disorder, you depend on that diagnosis to be based on facts.

For 22 years the *Diagnostic and Statistical Manual of Mental Health Disorders*, fourth edition (DSM-IV), with its 300 diagnoses has been the clinically accepted therapist's bible. It's routinely used to assess and determine patient diagnosis in the mental health field. Even so, a recent study from two Vanderbilt researchers has found therapists' objectivity can be clouded by personal beliefs. And that clouding can impact the diagnosis they attach to you and the kind of care you receive.

It's a finding Woo-kyoung Ahn, associate professor of psychology at Vanderbilt, and co-researcher Nancy Kim, a visiting faculty member from Wesleyan University, have uncovered in their work. Their results were published in December in the *Journal of Experimental Psychology.*

"Clinical psychologists have been told they should make their diagnoses based solely on a checklist of symptoms. But our results [find that they] are significantly more likely to diagnose patients with a mental disorder when the person exhibits symptoms that are central in the clinician's own theories. Similarly, they are far less likely to make the same diagnosis for a patient with symptoms they consider to be peripheral [to their own theories]," says Ahn. She and Kim hope their findings will have an impact on future editions of the DSM. Funding for their work came from the National Science Foundation and the National Institute of Mental Health.


Woo-kyoung Ahn

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In their study, Ahn and Kim had 35 clinicians and 25 clinical trainees perform four tasks. They measured participants' theoretical views by having them draw relationships between the symptoms of some disorders. Next, the subjects identified the relative importance of symptoms associated with disorders, and then they diagnosed hypothetical cases. Several hours later they tested participants' memories of the symptoms of the patients they diagnosed.

The researchers found the test group held complicated theories about various disorders—ranging from schizophrenia, major depression and anorexia nervosa to a variety of personality disorders—and the relative importance of various symptoms. Concurrently, the individuals' personal theories varied greatly.

In general, such theorizing appears to be part of human nature and is not necessarily bad, says Ahn. At the same time, because there is no basic understanding of the underlying causes of mental illness, the clinicians' individual theories can be idiosyncratic and lead to conflicting diagnoses.

And there's more. Ahn and Kim found individual theories held by clinicians about a given disorder affected the diagnoses. This, in turn, influenced the clinicians' recollection of patients' symptoms. They observed the clinicians and students were more likely to remember symptoms correctly if they judged them to be central to a given disorder. On the other hand, they were far more likely to forget symptoms they considered peripheral.

Even more striking was how clinicians' theories affected their memories of patients' symptoms. When they diagnosed patients with a specific disorder who did not have some of the symptoms that the clinicians considered central, they were likely to remember that the patients had these key symptoms when, in fact, they did not.

Ahn predicts these problems will be even more pronounced when clinicians are dealing with real patients. "I think this effect may actually be stronger because many more ambiguities surface when working with actual patients. For instance, clinicians' theories may influence their interpretation of patients' symptoms or characteristics, such as mood or level of hygiene."

Robot Fridays

3. "I sense that you are anxious. Is there anything I can do to help?"

No, it's not your therapist or your mother; it's your robot asking the question. There's no point in telling this robot "No, I'm fine," because your heart rate and sweaty palms have already given the game away. And your robot is right: Your anxiety could well signal that something important is going wrong, which could be extremely crucial information if you're working underwater or out in space.

Emotionally sensitive robots that can interpret the body's physiological signals would enjoy obvious advantages in being able to respond more rapidly and helpfully to situations that are confronting their human masters. This was the motivation that inspired one of the robotics laboratories at the Vanderbilt University School of Engineering to develop a robot that can mine physiological data from sensors attached to the body in order to interpret emotional state, treat it as important information, and respond to human needs quickly and usefully.

"There is a lot of communication that is implicit between two persons," says Assistant Professor of Mechanical Engineering Nilanjan Sarkar. "We study each other's faces and body language to see how the other person reacts." Other robotics researchers have used cameras to capture visual clues to emotion, but Sarkar wanted to focus on physiological manifestations of emotion, such as heartbeat.

Like most engineering problems, it turns out to be complicated. For one thing, individual human beings don't have the same physical reactions to their emotions. There is a great deal of individual variation; some people make facial gestures while others' heart rates skyrocket. Any emotion-sensing robot must be able to read the emotions of its particular human; universal patterns of emotional response don't exist.

For another, it turns out that researchers in different disciplines speak slightly different procedural and conceptual languages, making cross-disciplinary collaboration more challenging than either professor expected.

Sarkar had read a great deal about physiological responses to emotions and knew the robot would need to be able to learn its individual human's response patterns. He also knew that he would need to find a collaborator in psychology to help him design the human-subject research.

Vanderbilt Associate Professor of Psychology Craig Smith admits he was prepared to reject Sarkar's idea until he realized the engineer had done his homework in psychology and had come up with an intriguing idea. A specialist in cognitive studies at Peabody College, the project interested him. Learning about robot cognition might shed light on human cognition.

Pooling their academic expertise and resources, the two professors began to learn some tricks of the other's trade.

"It was a little bit of a culture shock for both of us," Smith says. "Engineering focuses on getting something to work. Research in psychology focuses on testing a hypothesis to infer causality."

"One of our biggest challenges was to enable the robot to extract data in real time so that it would not be lost forever in thought," Smith says. "The tools and techniques we used to extract and analyze the data quickly will also be applicable to many other psychology research projects."


Graduate student Pramila Rani wears emotion sensors that provide data to the robot about her moods.

Nilanjan Sarkar and Craig Smith

PHOTOS BY DANIEL DUBOIS

In their research, the professors used heartbeat, facial-muscle movement and hand-sweat sensors on human subjects and monitored these physiological markers while the subjects played video games. The research demonstrated some patterns of stress response when analyzed with wavelet analysis and fuzzy logic. They liken their approach to that used by voice and handwriting recognition systems: gathering baseline information about each person and analyzing it to identify the responses associated with different mental states.

The preliminary concepts and results of the research were reported in the *Robotica Journal,* published by Cambridge University Press. The article touched off a flurry of general science and news media articles and broadcasts on the professors' research.

Since then, the research team has taught the robot to decide whether to protect itself, sound alarms, or move to assist the person.

Future research will involve additional sensors such as electroencephalogram (EEG) brainwave monitors and additional measures of cardiovascular activity. One of the most formidable challenges they face is to find a way to discriminate between high levels of anxiety and engagement. These two states are accompanied by physiological responses that are much closer to each other than either of them are to low levels of anxiety or engagement.

"This is the really big one," Smith says.

High-Intake Oxygen Can Be Harmful

Oxygen bars have sprung up in malls across the country.

AP WIDE WORLD PHOTOS

At health spas, mall kiosks and "oxygen bars" across the country, people are paying to breathe oxygen. For about a dollar a minute, enthusiasts inhale 95 percent oxygen — air offers a paltry 21 percent O^2 — and report that it relieves a variety of maladies from hangovers to headaches.

The practice may be a bad idea, according to Vanderbilt University Medical Center scientists who are studying the damaging effects of free radicals—highly reactive molecules derived from oxygen.

"We're starting to think that oxygen is not as benign as many believe it is," said Dr. L. Jackson Roberts II, professor of pharmacology and medicine. Roberts and Joshua P. Fessel, an M.D./Ph.D. student, have discovered a new class of compounds, called isofurans, which form when free radicals attack cell membrane lipids. Isofurans, whose production is favored by high oxygen concentrations, are expected to be a useful tool for assessing the role of free radicals and oxidative injury in disease and for evaluating the effectiveness of antioxidant therapies.

Already, the investigators have demonstrated that isofuran levels increase when animals breathe 100 percent oxygen for as little as three hours. These findings, part of the group's work reported in the *Proceedings of the National Academy of Sciences,* demonstrate that free-radical processes are at work in hyperoxia-induced lung injury. "We suspected this to be the case, but we didn't have the tools to show it until now," says Roberts.

Hyperoxia-induced lung injury is a key problem in intensive care units. Patients on ventilators can only breathe oxygen concentrations up to 60 percent for prolonged periods of time. Higher concentrations — though of potential benefit to the body's organs — lead to severe lung damage.

The ability to measure isofuran production will make it possible to study the oxygen-induced damage and to evaluate potential therapeutic interventions like antioxidants, the researchers report.

"The question is, is there something we can do that would allow clinicians to actually use higher concentrations of oxygen safely, and therefore better oxygenate patients who are sick?" asks Roberts. "We don't know yet, but now we have a way to monitor that."

The fact that isofuran levels increased in the lung after only three hours of exposure to 100 percent oxygen — indicating that free-radical damage is a very early event — surprised the researchers. They also found evidence for the release of a trigger for programmed cell death, cytochrome c, in the lung at three hours.

"Most physicians are certainly aware that extended periods of exposure to 100 percent oxygen is harmful, but three hours would not be considered an extended period of time," Fessel says. The short time frame of free-radical damage opens questions about potential damage to the lungs of patients who breathe 100 percent oxygen during surgical procedures and to the lungs of those "oxygen bar" enthusiasts.

For a healthy individual, any damage that results from breathing high concentrations of oxygen for a short time is likely to be insignificant and spontaneously repaired, Fessel says. "But what about the person who has some underlying infection or other problem in the lung?" he asks.

Roberts and colleagues, including Dr. Jeffrey Balser, the James Tayloe Gwathmey Professor and Chair of Anesthesiology, and Dr. Kenneth Smithson, assistant professor of anesthesiology, are launching a clinical study to evaluate how free-radical processes might impact lung function in surgery patients. The study could suggest that lower oxygen levels would be beneficial, say Roberts and Fessel, or that antioxidant interventions should be tested to prevent free-radical damage.

The newly identified isofurans are actually the second set of compounds Roberts and colleagues have linked to free-radical processes. The group's 1990 discovery of isoprostanes, prostaglandin-like products of free-radical injury, made it possible for researchers to detect and monitor free-radical reactions in human beings for the first time. Measuring isoprostanes quickly became the "gold standard in the field," says Roberts, and it has been used to implicate free radicals in disease processes ranging from atherosclerosis to neurodegeneration.

But isoprostanes are not perfect measures of free-radical processes. Because the formation of these compounds becomes disfavored when oxygen levels climb above 21 percent, they do not provide an accurate measure of free-radical reactions that occur in the presence of high oxygen concentrations. The isofurans overcome this limitation. High oxygen levels favor the chemical reactions that produce isofurans, making them useful indicators of free-radical damage in high-oxygen settings like hyperoxia-induced lung injury, as the investigators showed, and for other oxygen-associated disease states like retinopathy of prematurity.

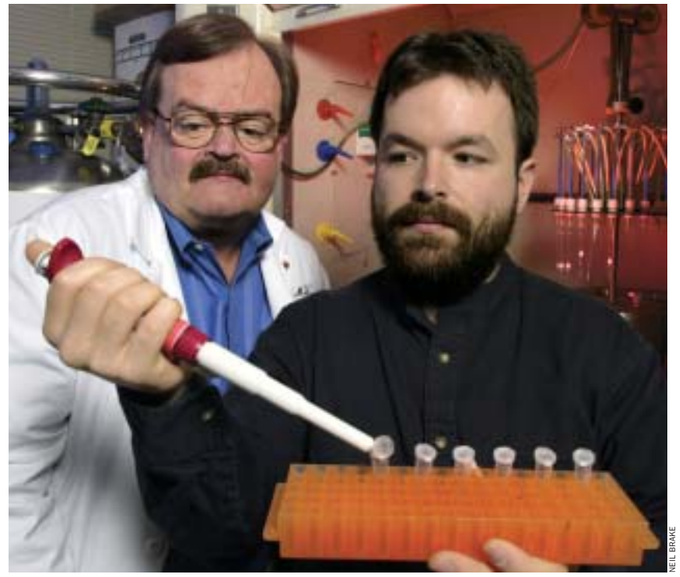
The investigators also have measured isofurans to assess oxidative injury in disease states involving mitochondrial dysfunction. Mitochondria — the power plants of cells — use oxygen in a complex series of energy-generating chemical reactions. They also generate free radicals. When mitochondria are not fully functional, oxygen levels inside the cell theoretically climb. Roberts and Fessel postulated that free-radical activity under these conditions might result in isofuran production.

Indeed, they found that isofuran levels were elevated in brain tissue samples from Parkinson's patients — Parkinson's disease is known to involve mitochondrial dysfunction — whereas isoprostane levels were unchanged. The investigators will continue to explore disease states where mitochondrial dysfunction is thought to play a role.

"Measuring isofurans really complements measuring isoprostanes," Roberts says. "Together the two of them provide a complete picture of oxidant stress."

The two also can serve as a sort of "oxygen sensor," say Fessel and Roberts. The researchers found that the ratio of isofuran to isoprostane concentrations in normal tissues — the compounds are produced by ongoing free-radical processes — provides an indication of tissue oxygenation. In oxygen-rich tissues like the brain and kidney, isofuran levels were two to three times higher than isoprostane levels. In the oxygen-poor liver, isoprostanes predominated.

"The isofuran/isoprostane ratio is really a measure of steady-state tissue oxygenation," says Fessel. The ratio should be useful for studying disease states where oxygen supply is perturbed, like peripheral vascular disease, or for assessing the effectiveness of so-called "blood substitutes" — compounds that carry oxygen to tissues, he says.



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Dr. Jackson Roberts (left) and Joshua Fessel have discovered a new class of compounds, called isofurans, which form when free radicals attack cell membrane lipids and whose production is favored by high oxygen concentrations. According to Roberts, "we're starting to think that oxygen is not as benign as many believe it is."

Other authors of the PNAS study include Ned A. Porter, Stevenson Professor of Chemistry; Dr. James R. Sheller, associate professor of medicine; and Dr. Kevin P. Moore of the Royal Free and University College Medical School in London. The work was supported by the National Institutes of Health and the PhRMA Foundation.

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