CREDIT: JOHN LAMB/GETTY IMAGES

**Science fiction?** Hydrogen sulfide might someday help people survive trauma and illness in a hibernation-like state.

MEDICINE

# Nothing Rotten About Hydrogen Sulfide's Medical Promise

**Despite its toxicity and famously bad odor, hydrogen sulfide's ability to lower metabolism and create a hibernation-like state has scientists wondering whether the gas can help soldiers and other people withstand injuries or surgeries**

It's foul-smelling, corrosive, flammable, and deadly. It's the bane of oil fields, sewage treatment plants, and farms because at concentrations workers sometimes encounter, a single breath of it can kill. Hydrogen sulfide ($H_2S$), the rotten egg gas, is not something you would think to pump into sick or injured people.

But that's exactly what some scientists plan to do, reflecting the gas's improving reputation over the past 2 decades. Long known for its distinctive smell and toxicity—it starves our cells by disabling an enzyme necessary for extracting energy from food—the molecule has proven to be an influential physiological signal, with effects on everything from blood flow to hormone secretion. Eager to capitalize on these newfound capabilities, scientists are trying to exploit hydrogen sulfide to tame the side effects of common painkillers, for example, and curb heart attack damage. After announcing last week that injecting low doses of hydrogen sulfide into healthy people produced no dangerous side effects, one company plans later this year to start testing the molecule as a treatment for several conditions, possibly including restricted blood flow to the liver and lung injuries. "We are right at the beginning of an expanding field that could have enormous clinical implications," says cardiovascular physiologist David Lefer of Albert Einstein College of Medicine in New York City.

In the past few years, hydrogen sulfide research has also veered into science-fiction territory, as investigators have found that nonlethal doses of the molecule can send small animals into a hibernation-like state. Whether this unexpected effect can be reproduced in large animals, or people, remains a matter of debate, but that hasn't stopped some scientists—and the U.S. military—from investigating whether the gas could allow patients to better survive severe injuries or traumas, such as a stroke, by placing them in a form of suspended animation.

### Not just sewer gas anymore

Toxicologists know plenty about the downside of hydrogen sulfide. Even at 10 parts per million (ppm), the exposure limit set by the U.S. National Institute for Occupational Safety and Health, the gas can irritate the eyes. If you breathe 500 ppm, you can die within half an hour, and 1000 ppm knocks you out instantly and kills within a few minutes.

So researchers were surprised to discover that the human body naturally makes the potentially lethal molecule, although at much lower concentrations. This ability represents a legacy from some of the earliest microbes, says physiologist Rui Wang of Lakehead University in Thunder Bay, Canada. These organisms—like a few living today—relied on sulfur, not oxygen, to obtain energy from metabolism. Although oxygen took over this job for the most part, organisms still put hydrogen sulfide to use for numerous other functions.

Hydrogen sulfide isn't the only noxious gas to reveal a good side. Nitric oxide was the first so-called gasotransmitter that scientists identified. Nobel Prize–winning work that started in the mid-1980s demonstrated that nitric oxide relaxes blood vessels, quells inflammation, nudges the hypothalamus to release hormones, and even transmits signals between the brain's neurons. Another killer gas, carbon monoxide, later joined the gasotransmitter family (*Science*, 21 November 2003, p. 1320). Despite these precedents, hydrogen sulfide researchers say they still get grief for their work, particularly from toxicologists. "A week doesn't go by when I'm not answering questions like, 'Isn't this a poison?'" says pharmacologist John Wallace of the University of Calgary in Canada.

Interest in hydrogen sulfide's possible benefits bubbled up about 20 years ago, when three papers described surprisingly high concentrations in brain samples from animals and humans. What was the molecule doing in healthy tissue, researchers wondered. It might help us to learn, replied neuroscientist Hideo Kimura and postdoc Kazuho Abe, then at the Salk Institute for Biological Studies in San Diego, California. In 1996, the pair found that in slices of rodent brain tissue, hydrogen sulfide spurs long-term potentiation, an increase in synapse sensitivity that can promote learning and memory. Unlike nitric oxide, hydrogen sulfide doesn't appear to transmit messages between neurons, says Kimura, who's now at the Institute of Neuroscience in Tokyo. Rather, it acts as a neuromodulator that





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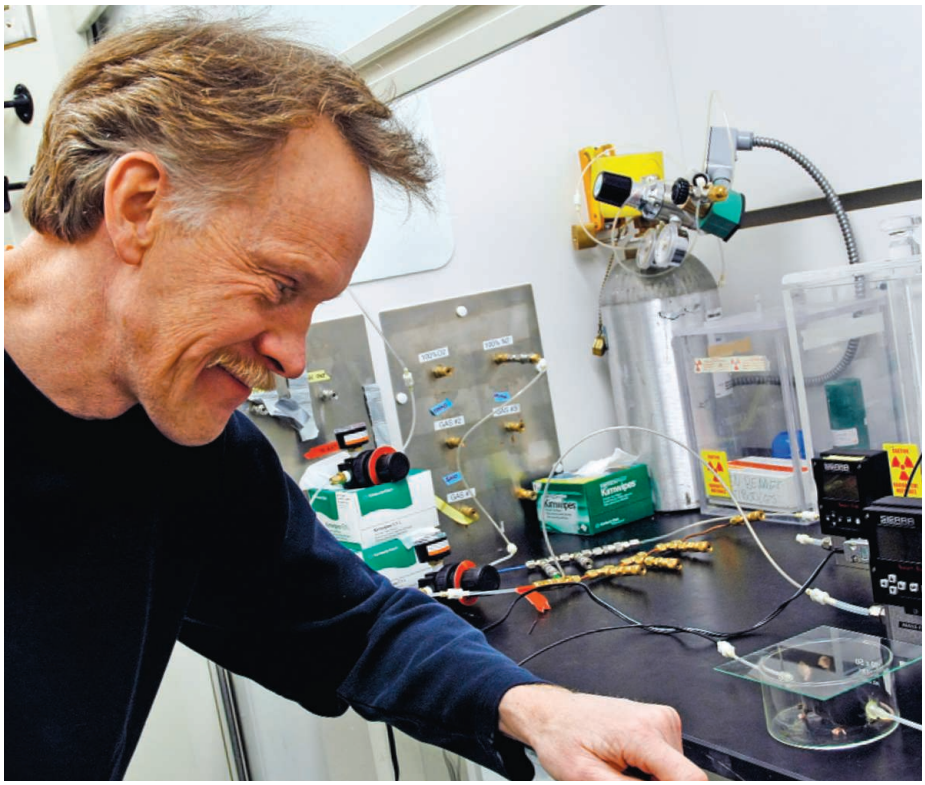


**Reanimator.** Mark Roth of the Fred Hutchinson Cancer Research Center prepares to give a mouse a dose of hydrogen sulfide gas.

adjusts the responsiveness of neural circuits.

The molecule's résumé includes other responsibilities, subsequent studies have shown. Many of its effects stem from the power to open the membrane channels that allow potassium to leave the cell, says Wang. For example, in 2001, his group showed that by prodding these channels, hydrogen sulfide relaxes smooth muscle cells in the walls of blood vessels, suggesting that the molecule regulates blood pressure. Researchers are still trying to resolve the sometimes contradictory reports on hydrogen sulfide's effects—for instance, studies conflict about whether it is pro- or anti-inflammatory—and nail down all of its functions. But physiologist David Kraus of the University of Alabama, Birmingham, predicts that hydrogen sulfide "will rival the prominence of nitric oxide."

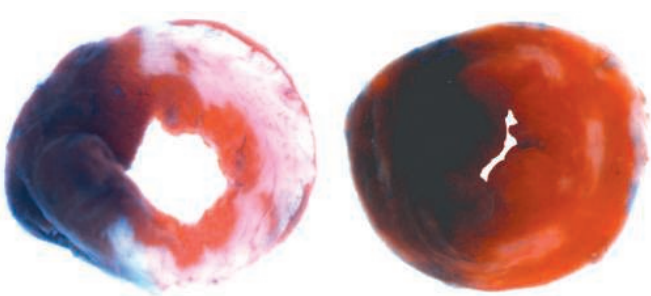
One apparent function of the molecule—soothing cells under stress—has attracted medical interest. Kimura and colleagues revealed 4 years ago that hydrogen sulfide can shield cultured neurons from oxidative damage, which often occurs after a stroke. Instead of combating oxidants directly, the molecule spurs cells to bump up the levels of glutathione, neurons' natural antioxidant. Researchers still need to test whether hydrogen sulfide curbs brain damage from a stroke in animals, Kimura says.

However, administration of hydrogen sulfide does seem to limit the damage from a heart attack, as Lefer and colleagues revealed last year. To simulate a clogged artery, the researchers temporarily tied off one of the vessels that delivers blood to the left ventricle in mice. An injection of hydrogen sulfide directly into the heart cut the amount of scarring and inflammation that resulted once blood flow resumed. Protection of mitochondria, the cell's energy-generating organelles, might explain that finding. In the untreated mice, the organelles' capacity to use oxygen plummeted. The mitochondria were swollen, and their complex interior structure appeared scrambled. This damage was absent in treated mice. Lefer and colleagues are already assessing hydrogen sulfide for other conditions, including heart failure, and plan to test sulfide-releasing pills that are now under development.

Pills that promote the creation of hydrogen sulfide might also protect the gut. Cells of the gastrointestinal (GI) tract naturally make the molecule, possibly to regulate blood flow or shield intestinal linings. In rodent studies, Wallace and his colleagues have found that intravenous administration of hydrogen sulfide fends off side effects, such as GI bleeding and ulcers, frequently caused by nonsteroidal anti-inflammatory drugs (NSAIDs), the drug class that includes aspirin and ibuprofen. Seeking easier-to-stomach NSAIDs, the researchers designed versions that emit small amounts of hydrogen sulfide. In tests on rats, the modification almost eliminated intestinal injury from the NSAID diclofenac, the team reported last year. These new NSAIDs "don't cause any GI damage at all and are as potent as the parent drugs," Wallace says. He predicts that the drugs will reach clinical trials within 18 months. And a sulfide-delivering version of mesalamine, a treatment for inflammatory bowel disease that causes similar GI anguish, could be ready for testing later this year, he says.

## A big sleep

While researchers have been gradually unearthing the physiological roles of hydrogen sulfide, Mark Roth's heart-slowing experiments with the gas grabbed headlines 3 years ago. A physiologist at the Fred Hutchinson Cancer Research Center in Seattle, Washington, Roth was studying how animals drastically reduce their metabolism, as some do during hibernation. His lab found that small animals such as nematodes and zebrafish embryos could endure oxygen concentrations supposedly below the minimal level for survival. The animals remained barely alive—the embryonic fish's hearts often stopped beating—but revived when researchers cranked up the oxygen levels. The team then started looking for ways to induce the same effect by preventing animals from using available oxygen. Carbon monoxide worked for nematodes, but they thought it would be too risky for humans.



**Heart healthy.** After a simulated heart attack, the scarred heart of a control mouse (*left*) contrasts with the ruddy tissue of a mouse treated with hydrogen sulfide (*right*).

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Roth says he decided to try hydrogen sulfide instead after seeing a TV documentary that mentioned its dangers to cavers.

When Roth's team exposed mice to 80 ppm of the gas, which toxicology studies had established was safe for rodents, the animals passed out. Their core body temperature plunged more than 20°C, their oxygen consumption fell, and their carbon dioxide output—an indicator of metabolic rate—tumbled. Once the researchers shut off the gas and provided heat, the mice were up and gnawing within an hour. They passed a battery of behavioral tests, indicating that they incurred no brain damage during their down time. In a follow-up study published last year, Roth's team showed that hydrogen sulfide could enable mice to survive low oxygen concentrations that are otherwise lethal to rodents.

After being gassed, the mice slip into an altered state that differs from the unconsciousness of sleep, hibernation, and anesthesia. Their eyes are closed, but unlike anesthetized patients, they are not paralyzed and respond to pain, says Warren Zapol, chief of anesthesia at Massachusetts General Hospital in Boston, who has studied the effects of hydrogen sulfide on the rodents. "You couldn't take their appendix out." he jokes. Pinch a tail, and they try to wriggle away, Zapol says. The animals aren't hibernating, either, as that involves much more gradual changes in everything from fat deposition to protein synthesis.

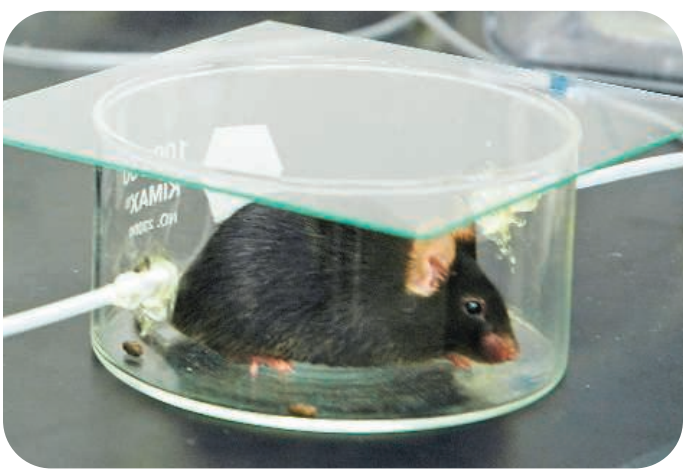
In an April paper in *Anesthesiology*, Zapol and colleagues provided the most detailed view yet of the cardiovascular changes induced by hydrogen sulfide. After breathing 80 ppm of gas, the animals' heart rate declined more than 50%, to about 250 beats per minute, the team reported. Typically, when a mouse's heart rate plunges, the organs can begin to run short of oxygen, Zapol notes. But the rodents' blood pressure remained steady, suggesting that the delivery of oxygen throughout the body didn't falter, he says.

hydrogen sulfide appears to serve as a master metabolic regulator, Roth says. He notes that 18th century British chemist Joseph Priestly likened humans to burning candles, consuming oxygen to keep the flame going. "We have stumbled across the mechanism by which human beings are regulating the degree to which they burn their candle," Roth says.

## Scent of survival

Roth now wants to lower the flame on people's candles. He envisions temporarily turning down metabolism and oxygen demand with a dose of hydrogen sulfide, buying time for patients who have suffered heart attacks, strokes, or wounds that produce drastic blood loss. In a study that *The Journal of Trauma* will publish in July, Roth and colleagues show that 66% of rats injected with a hydrogen sulfide solution can survive the loss of 60% of their blood, versus 14% of rats given a control solution. He's now testing for the same benefits in larger animals and has received a grant from the U.S. Defense Advanced Research Projects Agency to design injectable hydrogen sulfide kits that troops could carry in the field. Wounded soldiers could be "deanimated," suggests Roth, until they can be evacuated to a hospital.

Roth acknowledges that using hydrogen sulfide in this way faces some public relations hurdles. People expect doctors and paramedics to resuscitate their loved ones, not put them into a near-death state, he says: "The notion that you are better off deanimated than animated is not something people want to think about."



**Gassing up.** As this mouse breathes hydrogen sulfide, its heart rate, metabolic rate, and body temperature will plummet.

CREDIT: FRED HUTCHINSON CANCER RESEARCH CENTER

The bigger problem, say some critics, is that hydrogen sulfide won't elicit the same effect in people as it does in mice. Two studies on large animals bolster their skepticism. Last fall, for instance, a French team reported no metabolic decline in sheep that breathed 60 ppm of hydrogen sulfide gas.

Recent work on pigs by cardiologist Andrew Redington of the Hospital for Sick Children in Toronto, Canada, and colleagues suggests that nonlethal concentrations of the gas could exacerbate patients' problems. In their experiment, piglets that had been anesthetized much like a surgical patient breathed gradually increasing concentrations of hydrogen sulfide gas, from 20 ppm to 80 ppm. Instead of slowing the metabolism of the piglets, hydrogen sulfide revved it up, the team reported in January. Heart rate, blood pressure, and cardiac output were all higher in animals that breathed the gas than they were in controls. Many of the target patients for hydrogen sulfide treatment have weakened hearts, and forcing the organ to work harder could kill them, says Redington. His take is that in large animals, hydrogen sulfide "has no effect at best but possibly a detrimental effect." Resuscitation researcher Samuel Tisherman of the University of Pittsburgh Medical Center in Pennsylvania isn't as harsh but concedes that "some of the promise seems to be slipping away."

Roth counters that both large-animal studies share a crucial flaw: The concentration of hydrogen sulfide was too low. Although 80 ppm will knock out a mouse, a pig or sheep requires a bigger hit, he claims. He and his colleagues are conducting their own tests on large animals, but he says they're not ready to discuss the results.

Hydrogen sulfide doesn't need to produce dramatic effects to provide benefits, Roth notes. Ikaria, the company that he co-founded 3 years ago and for which Lefer serves as a consultant, has just announced the results of the first safety trial of an injectable form of hydrogen sulfide. Last week, the company revealed that 36 volunteers who received doses well below the level that causes unconsciousness in people showed no ill effects. Csaba Szabo, Ikaria's chief scientific officer, says that before the end of the year, the company plans to launch phase II trials. What types of patients will receive the drug isn't certain, but they could include people who have suffered heart attacks or who are undergoing operations such as a heart or lung bypass.

If suspended animation isn't futuristic enough for you, Roth published a paper last year hinting at another dramatic effect: increased longevity. His lab found that exposure to the gas stretches the life span of nematodes by up to 70%. Whether or not other organisms react the same way, it's clear that hydrogen sulfide has come a long way from just being a killer.

**–MITCH LESLIE**





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