Toxic Gas, Lifesaver

Hydrogen sulfide, a lethal gas best known for smelling like rotten eggs, turns out to play key roles in the body—a finding that could lead to new treatments for heart attack victims and others

By Rui Wang

Imagine walking into a hospital emergency room, with its hand-sanitizer-adorned walls and every surface meticulously scrubbed free of contaminants, only to encounter the stench of rotten eggs. Distasteful though this juxtaposition might sound, the toxic gas synonymous with that smell—hydrogen sulfide (H_2S)—may well become a fixture in such settings in the future. Over the past decade scientists have discovered that H_2S is actually essential to a number of processes in the body, including controlling blood pressure and regulating metabolism. Our findings indicate that if harnessed properly, the gas could, among other benefits, help treat heart attack patients and keep trauma victims alive until they can undergo surgery or receive a blood transfusion.

A Whiff of Poison

Scholars have known about H_2S's toxic effects on humans for centuries. Today it constitutes the number-one occupational safety hazard at oil and gas field wellheads, along pipelines, in processing plants and in refineries. Our noses can detect H_2S at concentrations of 0.0047 part per million (ppm). At 500 ppm, it impairs breathing. Exposure to 800 ppm for five minutes leads to death. Yet, paradoxically, we need H_2S to survive.

To see how the human body came to rely on this malodorous gas, page back some 250 million years to a time when the outlook for life on earth was very grim indeed. The Permian era was drawing to a close, and the single most devastating extinction event of all time was under way. Back then, carbon dioxide emissions from massive volcanic eruptions in Siberia triggered a chain of environmental changes that left oxygen levels dangerously low in the world's oceans and, according to a leading extinction theory, was ultimately responsible for the die-off [see "Impact from the Deep," by Peter Ward; SCIENTIFIC AMERICAN, October 2006].

This shift in ocean chemistry was bad

KEY CONCEPTS

- The body manufactures tiny quantities of the poisonous gas hydrogen sulfide (H_2S).
- Mounting evidence indicates that the gas plays a beneficial role in the health of the cardiovascular system and other parts of the body.
- Based on these findings, researchers are developing H_2S-based therapies for conditions ranging from cardiovascular disease to irritable bowel syndrome.

—The Editors

JAMIE CHUNG

news for oxygen-breathing, or aerobic, marine species. But anaerobic organisms known as green sulfur bacteria flourished under the low-oxygen conditions. The success of these bacteria made the ocean even more inhospitable to most of its remaining aerobic inhabitants, because they generated vast quantities of hydrogen sulfide. Eventually, so the theory goes, the lethal gas in the ocean diffused into the air, wiping out plants and animals on land. By the end of the Permian extinction, 95 percent of marine species and 70 percent of terrestrial ones had perished.

The importance of H_2S in human physiological processes is probably a holdover from that long-ago time. The creatures that survived this catastrophe were the ones able to tolerate and, in certain cases, even consume hydrogen sulfide, and we humans have retained some of that affinity for the gas.

Follow Your Nose

Hydrogen sulfide is not the only noxious gas that has been found at work in the human body. In the 1980s researchers began to uncover evidence that nitric oxide (NO), also known as nitrogen monoxide, is made by the body in low concentrations, where it functions as a signaling molecule, influencing cell behavior. In work that would garner the 1998 Nobel Prize in Physiology or Medicine, this nitric oxide was shown to dilate blood vessels, regulate the immune system and transmit signals between neurons, among other functions. And carbon monoxide (CO), a colorless and odorless gas often called "the silent killer," has similar effects.

Having studied CO and NO, I was convinced that the body probably made and used other such gasotransmitters. By 1998 I was constantly brainstorming about what those gases might be. That summer an idea came to me. After a busy workday, I came home to find a stinky smell in the house. I eventually traced the source to a glass cabinet where all my family's treasures were exhibited. The smell was emanating from a cracked and rotten egg, one of many Easter eggs my older daughter had painted as a school project. It was then that I started to wonder whether this rotten-egg gas, hydrogen sulfide, was also produced by our body's organs and tissues.

Because my work on CO and NO had focused on their effects on the cardiovascular system, I decided to begin my search for H_2S there, too. It was a good place to start: a series of experiments revealed significant activity.

The initial tests I conducted with my colleagues quickly revealed small quantities of the gas in the blood vessel walls of rats. Because rodent physiology is very similar to that of humans, the discovery meant human vessels undoubtedly made it as well. This finding was encouraging, but to determine whether H_2S is important to the functioning of the body, we were going to have to demonstrate far more than its mere presence in vascular walls.

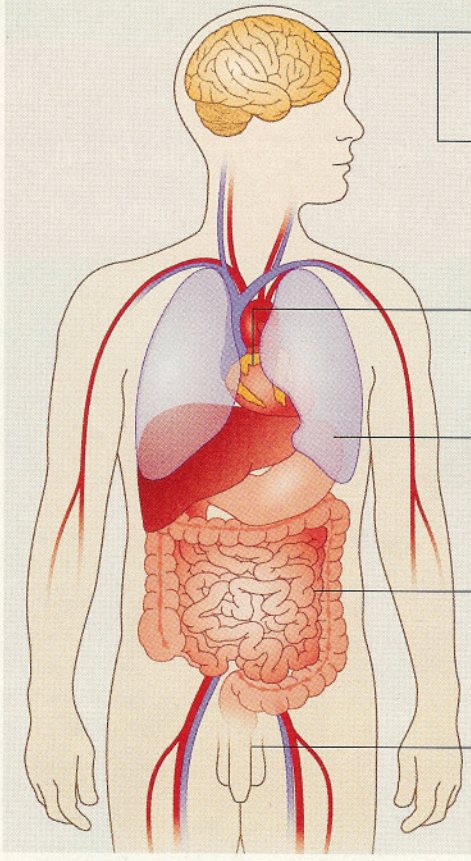
Findings suggest that H_2S could be used to prevent or treat hypertension, heart attacks and strokes in humans.

The next step was to figure out how the body makes H_2S. We decided to look at an enzyme called cystathionine-gamma-lyase (CSE), which was known to help produce the gas in bacteria. Previous studies had documented the presence of CSE in the liver, where it coordinates the construction of several amino acids, or protein building blocks, that contain sulfur. But no one knew whether CSE existed in blood vessels. Sure enough, we found the enzyme there, where it was combining with an amino acid called L-

[DISCOVERY]

A VITAL VAPOR

Scientists have determined that although hydrogen sulfide (H_2S) is toxic, it is actually made in small amounts in the body and may contribute to health in a number of ways, a representative selection of which is listed below. Not all the effects are beneficial, however: for example, too much H_2S can stymie the production of insulin, and some evidence suggests that it may worsen inflammation.



BRAIN
Increases the responsiveness of neural circuits, thus potentially aiding learning and memory

Promotes the production of the antioxidant glutathione, possibly protecting stressed neurons

CIRCULATORY SYSTEM
Dilates blood vessels, controlling blood pressure and protecting the heart

LUNGS
May help regulate contractility of smooth muscle cells in the lungs, opening airways

INTESTINES
Relaxes smooth muscle cells in the small intestine, regulating movement of material through the gut

PENIS
Relaxes penile tissue, helping blood to flow in and produce an erection

TAMI TOLPA

cysteine to produce H_2S and two other compounds, ammonium and pyruvate.

Having established the source of H_2S in blood vessels, we could turn our attention to unraveling its role there. Because NO had been shown to relax the blood vessels, we reasoned that H_2S might serve a similar purpose. Subsequent experiments bore that hunch out: when we soaked rat blood vessels in a H_2S solution, they dilated.

It was starting to look as though H_2S, like NO, was a regulator of blood pressure. The molecular mechanism for this phenomenon was still unknown, however. Hints eventually came from our studies of single cells taken from animal blood vessels. The results, which we published in 2001, proved surprising. Whereas NO relaxes blood vessel walls by activating an enzyme called guanylyl cyclase that resides in smooth muscle cells, H_2S manages the same feat through an entirely different pathway. Specifically, H_2S activates proteins called K_{ATP} channels that control the flow of potassium ions out of smooth muscle cells. This flow generates an electric current that limits the amount of calcium ions that can enter the cells, a constraint that relaxes the muscles and dilates the vessels.

Progressing from the isolated cells to living animals, we injected rats with a H_2S solution and found that their blood pressure dropped—presumably because the gas opened up the arteries, facilitating the flow of blood. We had mounting evidence that H_2S relaxes blood vessels, thus participating in blood pressure control. But we could not be sure that our addition of the gas to the blood vessels was truly replicating what happens when the vessels make their own H_2S.

To better gauge the effects of the gas, in 2003 my colleagues and I developed a line of mice engineered to lack the CSE enzyme and, hence, the ability to make H_2S in the blood vessels. We spent the next five years collaborating with research teams led by Solomon Snyder of Johns Hopkins University and by Lingyun Wu of the University of Saskatchewan in Canada to study these so-called knockout mice. Our efforts paid off, and in 2008 we published a paper in *Science* detailing our findings. As the modified mice aged, their blood vessels contracted, and they developed significantly higher blood pressure than is normal (as measured by tiny blood pressure cuffs fitted around their tails). When we injected the mice with H_2S, however, their blood pressure lessened.

The work with the knockout mice established beyond a doubt that hydrogen sulfide plays a vital role in the cardiovascular system. It also elucidated a longstanding mystery. For years after the Nobel Prize–winning work on NO, investigators had known that not all blood vessel dilation could be attributed to that gasotransmitter. For one thing, in animals genetically engineered to not produce NO in the endothelial cells that line vessel walls, peripheral blood vessels (those that do not lead directly to or from the heart) could still relax. But what could be causing that relaxation in the absence of NO?

Our studies indicate that the mystery factor is likely H_2S. Although we initially showed that the H_2S-generating enzyme CSE occurs in smooth muscle cells, subsequent studies of endothelial cells obtained from mice, cows and humans revealed that they, too, contain CSE—and in even greater quantities than the smooth muscle cells do. Exactly how the vessel-relaxing responsibilities are divided between NO and H_2S remains unclear, although some evidence suggests that NO does most of the work in large vessels and that H_2S takes over in small ones.

A Pound of Cure?

The revelation that H_2S is produced in the cardiovascular system and helps to control blood pressure caught the attention of many other researchers who had been looking for novel ways to protect the heart against damage from oxygen deprivation, as occurs when a clot prevents blood from bringing oxygen to the heart, leading to the death of cardiac tissue (a heart attack). In 2006 Gary F. Baxter, now at the University of Cardiff in Wales, and his colleagues reported that in isolated rat hearts, which were first provided with saline solution to mimic blood supply and then deprived of the saline to mimic a heart attack, administering H_2S to these isolated hearts before halting the saline supply reduced the extent of cardiac muscle damage. And David Lefer of Emory University showed the following year that mice engineered to produce more H_2S in the heart were better able to tolerate oxygen deprivation caused by a clot and more resistant to the damage that often ensues when blood flow is restored to tissues after a period of deprivation (a condition known as reperfusion injury).

Findings such as these suggest that H_2S could be used to prevent or treat hypertension, heart attacks and strokes in humans. But the gas's ability to relax blood vessels means that its potential applications extend to other blood vessel prob-

WHY GARLIC IS GOOD FOR YOU

Studies suggest that garlic can soften blood vessel walls, prevent blood platelets from clumping together and lower blood pressure, thus lowering the risk of heart attack, stroke and kidney disease. Research has also linked eating garlic to improved immune system function and reduced risk of some forms of cancer.

The secret of garlic's apparent health benefits may lie in its relation to H_2S. In 2007 David W. Kraus of the University of Alabama reported that the sulfide-containing compounds found in garlic are converted into H_2S by molecules that occupy the membranes of red blood cells. Furthermore, garlic contains a compound called S-allyl-L-cysteine that boosts the production and circulation of H_2S in the body, according to findings published that same year by Yizhun Zhu of Fudan University in Shanghai and his colleagues.

[THE AUTHOR]

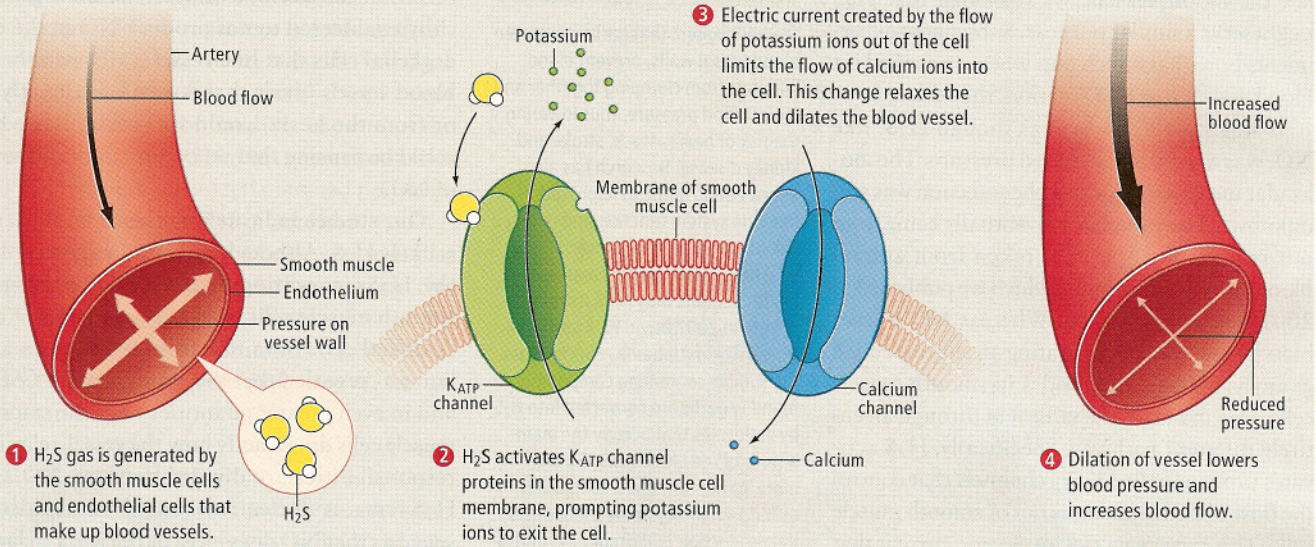
Rui Wang is a professor of biology and vice president of research at Lakehead University in Thunder Bay, Ontario. He is also president of the Canadian Physiological Society and a leader in the study of the metabolism and physiological functions of a group of small molecules of gas known as gasotransmitters, including nitric oxide, carbon monoxide and hydrogen sulfide. In 2008 Wang received the prestigious Pfizer Senior Scientist Award from the Canadian Society of Pharmacology and Therapeutics. He is an avid NBA basketball fan and loves science-fiction movies.

BRAND X PICTURES *Getty Images (garlic)*; COURTESY OF RUI WANG *(Wang)*

[HOW H_2S WORKS]

RELAXING GAS

Hydrogen sulfide plays a key role in regulating blood pressure. Earlier studies had shown that another gas, nitric oxide, relaxes blood vessels by activating an enzyme called guanylyl cyclase located in the vessels' smooth muscle cells. Recently scientists have determined that H_2S has the same dilating effect on the vessels, but it acts through a completely different pathway, shown here.



lems, too—including erectile dysfunction. Penile erection is maintained by the dilation of blood vessels. In fact, Viagra works by prolonging the effect of NO in the penis, where the gas acts to relax the vessels, thereby enhancing blood flow. Studies suggest that H_2S could produce the same effect, although more work is needed to determine its exact role in human penile tissue. (CO, too, is produced in the penis, although it facilitates ejaculation, not erection.)

H_2S is not confined to the cardiovascular system. It is also made in the nervous system, though not by CSE but rather an enzyme known as cystathionine beta synthetase. Exactly what the gas does there is uncertain. Some studies suggest that it is a neuromodulator, making neural circuits more or less responsive to stimuli. It may participate in a process called long-term potentiation that facilitates cell communication and may thereby promote learning and memory. In addition, the gas has been shown to increase levels of the antioxidant glutathione in neuronal cells, suggesting that it protects these cells against stress. And it may help the body to sense pain so that it can react accordingly.

Moreover, the gas seems to help regulate metabolism—the chemical processes that manage energy use and synthesis in the body. In a stunning series of experiments, Mark B. Roth of the University of Washington and his colleagues administered low concentrations of H_2S to mice to decrease metabolism and thereby retard the progression of certain diseases. The animals' heart rate instantly dropped by half, sending them into a state of suspended animation in which metabolism slowed so much they were able to get by on an inhaled "diet" of H_2S and oxygen alone without obvious negative effects. During this "H_2S hibernation," it seems, the body maintains a baseline metabolism that protects the vital organs from damage until energy supply levels return to normal. Within 30 minutes of stopping H_2S inhalation, the animals resumed their usual metabolic rate [see "Buying Time in Suspended Animation," by Mark B. Roth and Todd Nystul; SCIENTIFIC AMERICAN, June 2005].

Whether H_2S hibernation can put life on hold while preserving critical brain functions, such as memory and reason, remains to be seen.

If proved to be effective and safe in humans, H_2S hibernation could be a boon for emergency medicine. Inhalation of H_2S at the site of a car accident or by a person experiencing a heart attack could conceivably buy the time needed to successfully transport a patient to the hospital for lifesaving treatment. H_2S could also conceivably keep a patient alive in a suspended state until a needed organ becomes available (the gas might also keep donated organs viable longer). Additionally, war zones and natural disaster zones could benefit from the availability of H_2S therapy, which could ease the demand for blood transfusions until a sufficient blood supply became available. In 2008 Roth and his colleagues reported that rats that inhaled H_2S after losing

TAMI TOLPA

60 percent of their blood fared far better than did rats that did not receive treatment, with only 25 percent of treated rats succumbing to the trauma as compared with 75 percent of the untreated rats.

Cautious Optimism

Yet not everything H_2S touches turns to gold. The jury is still out on whether the gas worsens or alleviates inflammation, for example. And studies in my laboratory and elsewhere suggest that the gas is a key player in type 1 diabetes, the kind that often occurs in childhood and leaves people dependent on insulin injections for survival. H_2S is produced in, among other places, insulin-producing cells in the pancreas called beta cells. In animals with type 1 diabetes, H_2S production is abnormally high in these cells. This surplus of the gas has two ill effects. First, it kills off a large number of beta cells, leaving behind too few to produce the insulin the body requires to break down glucose for energy. Second, it hinders the release of insulin from those remaining beta cells. In other words, H_2S may be partly to blame for the insufficient level of insulin in the blood in cases of type 1 diabetes.

Furthermore, some of the positive effects of H_2S documented in rats and mice have not been replicated in larger mammals. In a 2007 study conducted by a French team, for instance, sheep given the gas did not enter the quasi hibernation state seen in the rodents. And in another study, piglets that received H_2S showed an increase in metabolic rate rather than a decrease.

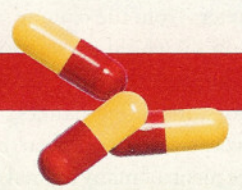
Neither is it clear whether H_2S hibernation, when it can be induced, impairs brain function. Although laboratory assessments have not detected such malfunctioning in treated animals, brain function changes are rather difficult to detect in experimental animals. It remains to be seen whether H_2S hibernation can put life on hold while preserving critical brain functions, such as memory and reason.

Nevertheless, the great therapeutic potential of H_2S has generated considerable interest in the pharmaceutical industry. Already several companies are developing products aimed at delivering doses of H_2S to the body. For example, CTG Pharma in Italy has generated various compounds that are hybrids of nonsteroidal anti-inflammatory drugs (called NSAIDs) and H_2S. Experiments in animals indicate that these drugs are effective in treating neuronal and gastrointestinal inflammation, erectile dysfunction, heart attack, and pathological changes to the structure of the blood vessels. Meanwhile New Jersey–based Ikaria, co-founded by Roth, recently launched phase II, or efficacy, trials of an injectable form of H_2S for people who have had heart attacks or are undergoing heart or lung surgery.

Despite people's natural inclination to avoid exposure to H_2S, it is clear from the research conducted over the past decade that this gas plays a critical role in the health of the heart and potentially in the brain and other organs. And it probably acts in other capacities that we have yet to identify. These breakthroughs will guide physiologists in developing a new conception of the molecular basis of human health. The ongoing work on hydrogen sulfide is still young, but chances seem good that it will eventually lead to treatments for some diseases whose cures have thus far eluded us. ■

[H_2S-BASED THERAPIES]

H_2S TO THE RESCUE



Drug developers are currently evaluating the potential of H_2S-based compounds for treating a number of conditions.

CONDITION	COMPOUND	COMPANY	STAGE OF DEVELOPMENT
▪ Heart surgery ▪ Heart attack ▪ Kidney injury	IK-1001	Ikaria	Phase II efficacy trial Phase II efficacy trial Phase I safety trial
Inflammatory bowel disease	ATB-429	Antibes	Phase I safety trial
Acute and chronic joint pain	ATB-429	Antibes	Preclinical
Irritable bowel syndrome	ATB-429	Antibes	Preclinical
Arthritis	ACS-15	CTG Pharma*	Preclinical

*The author will be testing compounds for CTG Pharma.

A KEY TO LONGEVITY?

Preliminary work hints that hydrogen sulfide may influence longevity. In experiments with the nematode worm *Caenorhabditis elegans,* Mark B. Roth of the University of Washington and his colleagues found that worms raised in an environment containing a low concentration of the gas in the air lived 70 percent longer than untreated worms. Curiously, H_2S does not appear to have acted via any of the three main pathways known to control longevity in these creatures. The mechanism by which the gas extended the worm life span remains uncertain, but it may regulate a gene called *sir-2* that has been linked to long life in worms and other organisms. The researchers detailed their findings in the *Proceedings of the National Academy of Sciences USA* in 2007.

MORE TO EXPLORE

Two's Company, Three's a Crowd—Can H_2S Be the Third Endogenous Gaseous Transmitter? R. Wang in *FASEB Journal,* Vol. 16, pages 1792–1798; November 2002.

H_2S Induces a Suspended Animation-like State in Mice. E. Blackstone, M. Morrison and M. B. Roth in *Science,* Vol. 308, page 518; April 22, 2005.

H_2S as a Physiologic Vasorelaxant: Hypertension in Mice with Deletion of Cystathionine Gamma-Lyase. G. Yang et al. in *Science,* Vol. 322, pages 587–590; October 24, 2008.

Pancreatic Islet Overproduction of H_2S and Suppressed Insulin Release in Zucker Diabetic Rats. L. Wu et al. in *Laboratory Investigation,* Vol. 89, pages 59–67; January 2009.

YO Getty Images (candles); IMAGE SOURCE Getty Images (pills)