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Abstract: The article discusses research to reversibly stop the human biological clock in order to safeguard the critically injured or preserve donor organs for transport. Nature abounds in organisms that can and do reversibly arrest their essential life processes. Scientists describe these phenomena by a variety of terms--quiescence torpor, hibernation--but all represent different degrees of suspended animation, a dramatic reduction of both energy production (metabolism) and energy consumption (cellular activity). Some human organs destined for transplantation, such as the heart and lungs, can survive outside the body for only up to six hours. If these precious organs could be placed in a suspended state, their viability might be preserved for days or even weeks. Studies in our laboratory at the Fred Hutchinson Cancer Research Center and by other researchers have shown that hibernationlike state can be induced on demand in animals that do not naturally hibernate. Animal research supports the idea that even in larger mammals, decreasing levels of available oxygen can prevent damage to tissues. We believe that hydrogen sulfide may be the key to safely inducing such suspended animation-like states. INSET: LIFE IN BALANCE.

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BUYING TIME IN SUSPENDED ANIMATION

An ability to put the human body on hold could safeguard the critically injured or preserve donor organs for transport. Does the power to reversibly stop our biological clocks already lie within us?

FANTASY WRITERS HAVE LONG BEEN CAPTIVATED BY the possibility of preserving human life in a reversible state of suspended animation. In fictional tales the technique enables characters to "sleep"

through centuries of interstellar travel or terrestrial cataclysms, then awaken unaffected by the passing of time. These stories are great fun, but their premise seems biologically far-fetched. In reality, we humans do not appear capable of altering our rate of progression through life. We cannot pause the bustling activity of our cells any more than we can stop breathing for more than a few minutes without sustaining severe damage to vital organs.

Nature, however, abounds in organisms that can and do reversibly arrest their essential life processes, in some cases for several years at a time. Scientists describe these phenomena by a variety of terms--quiescence torpor, hibernation, among others--but all represent different degrees of suspended animation, a dramatic reduction of both energy production (metabolism) and energy consumption (cellular activity). What is more, organisms in this state enjoy extraordinary resistance to environmental stresses, such as temperature extremes, oxygen deprivation and even physical injury.

Sci-fi scenarios aside, if the human body could be placed in such a condition, the implications for medicine alone would be enormous. For example, some human organs destined for transplantation, such as the heart and lungs, can survive outside the body for only up to six hours. Others, such as the pancreas and kidney, cannot last for more than a day. Successful organ transfers thus depend on speed, which means that in some cases potential matches must be passed over for a simple lack of time to transport the organ before it deteriorates. And while tens of thousands of organ transplants are performed successfully every year in the U. S., this urgency sometimes leads to mistakes that might have been averted had there been more time.

If these precious organs could be placed in a suspended state, their viability might be preserved for days or even weeks. Emergency medical teams could also use this technique to buy time for critically injured trauma victims. Putting these patients into suspended animation could stave off deterioration of their tissues while doctors repaired their injuries.

Recent studies in our laboratory at the Fred Hutchinson Cancer Research Center in Seattle and by other researchers have shown that hibernationlike state can be induced on demand in animals; that do not naturally hibernate. Moreover, such animals seem to be protected from the usual effects of blood loss, such as oxygen deprivation, while they are in a suspended state. These results raise the exciting possibility that suspended animation may be feasible in humans as well. Indeed, the methods our group has used to induce suspended animation in lab animals and in human tissue suggest this capability could be latent in many organisms through a mechanism with roots in the earliest days of microbial life on earth.

Survival of the Slowest

THE DIVERSE RANGE of creatures known to be capable of stopping some or most of their cellular activity usually do so in response to an environmental stressor and remain "stopped" until it is removed. A developing plant seed, for instance, can stay dormant in the soil for years until conditions favor germination. Similarly, embryos of a species of brine shrimp, Artemia franciscana, popularly known as sea monkeys, can live for more than five years without any food, water or oxygen by entering into a seed like state called quiescence, in which cellular activity is at a virtual standstill. On re-exposure to their natural environment, they will resume developing normally toward adulthood.

Suspended animation-like states can range from those in which animation is truly halted all movement within cells visible through a microscope stops to states where cellular activity continues but at a drastically slowed pace. A variety of adult animals, for example, can radically reduce their need for food and air over long periods by hibernating: their breathing and heart rate become almost imperceptible, their body temperature drops to near freezing, and their cells consume very little energy. Ground squirrels and dozens of other mammalian species pass the cold winter months in this condition every year, whereas other animals, including varieties of frogs, salamanders and fish, take refuge during hot summer months in a similar state called estivation.

The ability to survive even prolonged oxygen deprivation, which these organisms gain by dramatically reducing their need for and production of energy, offers a stark contrast to the normal situation of humans. We are thoroughly dependent on a steady supply of oxygen because our cells need it to maintain their constant production of energy. When oxygen levels within our tissues fall below a precise range, cells suffer ischemic damage, leading to tissue death. Thus, ischemia is often the underlying cause of mortality following heart attacks, strokes, or other physical traumas that deprive tissues of blood, and therefore oxygen, even if only for a short time.

Some of the molecular events that cause tissue damage in ischemia are not yet fully understood, but scientists certainly agree that cells' loss of their ability to fuel essential self-maintenance activities must play a central role. Most of the energy that cells consume comes from molecules of adenosine triphosphate (ATP), which are manufactured primarily by cellular mitochondria in an oxygen-dependent process known as oxidative phosphorylation. When oxygen levels drop, oxidative phosphorylation slows and ATP levels decrease. Because ATP molecules are typically consumed by a cell within seconds after they are produced, ischemic damage is believed to result when cells without sufficient oxygen simply run out of gas.

The damage may be worsened when some cellular processes that are less energetically demanding, but equally essential, continue, throwing a cell's overall system out of coordination. Finally, oxidative phosphorylation itself can also harm the cell. When oxygen levels fall below an optimal concentration, the oxidative phosphorylation process becomes less efficient and can release energy prematurely in the form of highly reactive molecules called free radicals. These by-products have become famous for their aging effects because they can damage DNA and other cellular structures. In ischemia, their actions further hinder an oxygen-deprived cell's ability to carry out crucial functions.

The goal, therefore, of CPR and other conventional approaches to preventing ischemic damage in victims of traumatic injury is to restore blood flow-and thus oxygen supply--to tissues as rapidly as possible. Given our cells' strict oxygen requirements, that might seem to be the only possible strategy. We have seen, however, that for animals in suspended animation-like states, dramatically reduced cellular activity makes them remarkably resistant to ischemia during oxygen deprivation. Suspecting that inducing the same condition in humans might enable people to avoid ischemic damage during periods of low oxygen, our group began working to understand more about the mechanism that allows organisms to shut down: in response to oxygen deprivation.

Lessons from a Worm

WE HAVE STUDIED suspended animation in a variety of popular laboratory workhorse organisms, such as yeast, zebrafish embryos and the sod nematode Caenorhabditis elegans. The last is able to enter a state of suspended animation at any stage of life. It will do so when placed in anoxia--an atmosphere with extremely low oxygen content of around 0.001 percent or less-and "can maintain this arrest for 24 hours or more.

When blood flow to human tissue is cut off, however, whether by blood loss or a vascular blockage, oxygen concentrations probably never drop low enough to make the tissue completely anoxic. Residual oxygen in remaining blood and in the tissue itself could allow low levels of oxidative phosphorylation to occur. But ATP production would be insufficient to support normal rates of cellular activity, and damaging free radical production would increase.

To mimic these ischemic conditions for humans, we can expose developing C. elegans embryos to "hypoxic" oxygen concentrations, between 0.01 and 0.1 percent O^2--still well below the 21 percent oxygen in normal room air (normoxia) but slightly higher than anoxia. In hypoxia, the embryos do not enter into suspended animation as they would in anoxia. Instead they attempt to continue their progression through embryogenesis, resulting in obvious cellular damage and death after 24 hours.

If we increase the oxygen concentration in the embryos' atmosphere just slightly, to 0.5 percent O^2, they

progress normally through embryogenesis just like embryos in normoxia. Thus, even though the nematodes are capable of surviving in anoxia by entering into suspended animation and Can develop normally in as little as 0.5 percent 02, the 10-fold range of oxygen concentrations between these two spaces is lethal.

We have also shown in our work with C. elegans that the embryos' shift into suspended animation under anoxic conditions is not merely a passive result of their running out of oxygen but rather seems to be a purposeful mechanism. We identified two genes functioning during anoxia, but not hypoxia, that appear essential to arresting the embryos' cell cycle. When exposed to anoxia, embryos lacking these genes fail to suspend their cell divisions, their chromosomes segregate improperly, and many die.

These results suggest that ischemic damage can be avoided not only by increasing the amount of oxygen available to cells, as conventional wisdom would predict, but also by decreasing available oxygen. This idea may fly in the face of current medical practice, yet it has strong implications for preserving human tissues: it is difficult to keep an individual organ destined for transplantation oxygenated or to supply enough oxygen to the damaged tissues of injury victims, but it might be possible to decrease their available oxygen.

One effective way to reduce a cell's access to oxygen is to add a mimetic--a substance that physically resembles oxygen at a molecular level and thus can bind to many of the same cellular sites but that does not behave like oxygen chemically. Carbon monoxide, for example, can compete with oxygen for binding to cytochrome c oxidase, a component of the oxidative phosphorylation machinery within the cell that normally binds oxygen, but the bound carbon monoxide cannot be used to produce ATP.

We therefore wondered if we could protect C. elegans embryos from the ischemic damage they faced in intermediate oxygen concentrations by simultaneously adding carbon monoxide to their hypoxic atmosphere--effectively simulating anoxia by blocking the small amount of remaining oxygen available to the embryos. Indeed, we found that under these conditions the embryos entered into suspended animation and avoided the lethal effects of ischemia.

By 2003 these encouraging results made us eager to test this concept further. Previous studies of larger animals and intriguing stories of human accident victims surviving conditions of low oxygen suggested to us the mechanism that rescued our worms might also exist in more complex organisms.

Inducing a Protective Pause

CONSIDERABLE ANIMAL research supports the idea that even in larger mammals, decreasing levels of available oxygen can prevent damage to tissues. When animals hibernate naturally, for instance, the suspended state appears to protect them from injuries. Experiments by Kelly L. Drew of the Institute of Arctic Biology at the University of Alaska Fairbanks and her colleagues found that when the brains of hibernating arctic ground squirrels were pierced with microscopic probes, little or no brain tissue died. The same injury inflicted on nonhibernating squirrels caused rapid tissue deterioration.

Such evidence has led several researchers to try to induce a hibernationlike state in animals that do not normally hibernate to see whether the cellular slowdown itself could be achieved safely and whether it might protect tissues long enough to repair an injury. The late Peter Safar and his co-workers at the University of Pittsburgh worked for nearly two decades with dogs to perfect a process for creating a state of suspended animation. Last year Safar's group described its most recent experiments. To create the suspended state, cardiac arrest was induced in each of 14 dogs, and then the blood was drained out of the animals' bodies while a cold saline solution was infused into them. Saline has a much lower capacity for carrying oxygen than blood, so this procedure dramatically reduces the amount of oxygen in the dogs' tissues. Afterward, the dogs were unconscious, did not breathe and had no heartbeat.

Safaris team then separated the dogs into a control group of six animals and a second group of eight that

would undergo surgical removal of their spleens, a nonessential organ. After 60 minutes in the suspended state, all the dogs were revived by reinfusion of blood. Seventy-two hours later the dogs were all still alive, and none of the control dogs showed any functional or neurological ill effects from their time in suspended animation. Four of the eight surgery dogs were also normal, although the other four displayed some neurological deficits.

Peter Rhee and his colleagues at the Uniformed Services University of the Health Sciences used a similar technique to induce suspended animation in 15 adult Yorkshire swine. They then performed vascular repair surgery on some of the animals. Rhee reported that the memory and learning abilities of all the test animals were completely unaffected by their experience.

Because the physiology of dogs and pigs is so similar to that of humans, this line of research has prompted enthusiastic speculation that such procedures could soon be perfected and tested on human patients in emergency rooms.

Although this method may hold promise, exsanguination is a drastic action with great potential for complications, so our group has been searching for less invasive ways to temporarily deprive living cells of oxygen. For instance, in blood-free human tissues, such as an organ that has been removed from a donor, suspended animation might be induced by placing the organ in an airtight container and perfusing the tissue with carbon monoxide, as we did with the C. elegans embryos. When doctor's were ready to implant the organ, they would need only infuse it with blood to restore its oxygen supply. In our lab, we have experimented with this technique to preserve human tissue samples from normal cellular deterioration, and we believe this approach could significantly extend the viability of human organs destined for transplantation.

The effects of carbon monoxide would be easily reversed in explanted organs, although the same would probably not be true in living organisms with blood coursing through their bodies. Because carbon monoxide molecules bind tenaciously to red blood cells at the sites where oxygen would normally attach itself, using this gas in trauma victims would be impractical. We have therefore also been experimenting with alternative oxygen mimetics.

Most of the substances we have tested are, like carbon monoxide, considered human poisons precisely because they can block cells' ability to use oxygen. Pockets of hydrogen sulfide gas, for example, are a deadly hazard to workers in many industrial settings, such as sewers and "sour gas" fields in the petrochemical industry. For this reason, occupational safety research has defined lethal doses of hydrogen sulfide (H_2S), largely through studies using rodents. This work provided a helpful starting point as we began to test nonlethal doses of H_2S on laboratory mice to see whether it could induce a reversible state of suspended animation.

In a sealed chamber, we exposed mice to atmospheres containing as much as 80 parts per million H_2S. At that level we observed a threefold drop in their carbon dioxide output within the first five minutes, and their core body temperatures began to fall. The animals ceased all movement and appeared to lose consciousness. Over the course of several hours in this environment, the animals' metabolic rate continued to decrease, as measured by their carbon dioxide output, ultimately falling 10-fold. Their breathing rate slowed from a norm of 190 breaths per minute to fewer than 10.

The animals' core body temperature kept dropping from their usual 37 degrees Celsius until it reached a level approximately two degrees C above the air temperature, regardless of what that was. We were able to bring their average body temperatures as low as 15 degrees C simply by cooling their chamber. In naturally hibernating animals, this same tendency of body temperature to rise or fall along with ambient temperature is common.

In effect, treatment with H_2S converted our mice from warm-blooded to cold-blooded, which is just what

happens to animals during hibernation. We kept the mice in this condition for six hours, and after they revived we gave them a battery of tests to see if their suspended animation experience had left any functional or behavioral ill effects. The mice all seemed perfectly normal.

From Mice to Men

WE ARE NOW continuing this line of research in larger animals, and we believe that hydrogen sulfide may be the key to safely inducing such suspended animation-like states in organisms that do not normally hibernate, including humans. Although H_2S is considered a poison, it is also produced naturally within our own bodies. Indeed, H_2S may have a unique unrecognized role in regulating cellular energy production in oxygen-breathing organisms because it once played oxygen's molecular part in metabolism when our planet was young and oxygen was scarce [see box on opposite page]. Many other questions still remain to be answered, however, before H_2S-induced suspended animation can be studied in people.

The biggest unknown is whether humans are even capable of entering a state of suspended animation. Compelling evidence certainly indicates that human beings are sometimes able to withstand several hours without oxygen. In one remarkable example just a few years ago, a Norwegian backcountry skier was rescued after an accident that left her under ice-cold water for more than an hour. When the emergency crew found her, she was clinically dead--not breathing, without a heartbeat, and with a core body temperature of 14 degrees C (57 degrees Fahrenheit). Despite requiring nine hours of resuscitation, she has since made an "excellent" recovery, according to her doctors.

Another 32 cases of severe hypothermia in which core body temperatures ranged from 17 to 25 degrees C (63 to 77 degrees F) and many of the victims lacked vital signs when rescued were analyzed by Beat H. Walpoth of the University of Bern in Switzerland. He found that nearly half--15 patients--recovered from the trauma without any long-term impairment.

Because these people were not breathing, oxygen levels in their tissues were undoubtedly very low, suggesting that, on occasion, the human body also possesses the flexibility to reversibly slow or stop cellular activity in response to a stress. But which occasions? What variables allow some people to live under these conditions, whereas others die? Understanding the links between natural and induced suspended animation in animals and the largely unexplained survival of certain human patients may reveal that the capacity to enter a protective state of suspended animation already exists within all of us.

Overview/Putting Life on Pause

• Many organisms are naturally able to slow or arrest their life processes, and their suspended state confers protection from environmental conditions that would normally kill them, such as prolonged oxygen deprivation.

• Inadequate oxygen is a major cause of tissue damage and death in explanted donor organs and in people experiencing blood loss or obstruction. Restoring oxygen supply to these tissues is not always immediately possible. Blocking all available oxygen, however can induce a variety of animals to enter protective suspended animation and might do the same for human injury victims or tissues.

• Hydrogen sulfide, a chemical produced naturally by our bodies, blocks cells from using oxygen and triggers suspended animation in mice, It may be a natural regulator of cellular energy production that could be employed to induce a protective suspended state in humans.

MORE TO EXPLORE

Ecology and Evolution in Anoxic Worlds. Tom Fenchel and Bland J. Finlay. Oxford University Press, 1995.

Oxygen: The Molecule That Made the World. Nick Lane. Oxford University Press, 2004.

Carbon Monoxide-Induced Suspended Animation Protects against Hypoxic Damage in Coenorhabditis elegans. Todd G. Nystul and Mark B. Roth in Proceedings of the National Academy of Sciences USA, Vol. 101, No. 24, pages 9133-9136; June 15, 2004.

Hydrogen Sulfide Induces a Suspended Animation-like State in Mice. Eric Blackstone, Mike Morrison and Mark B. Roth in Science, Vol. 308, page 518; April 22, 2005.

BEATING THE CLOCK

Organs become vulnerable to ischemic damage as soon as they are disconnected from their donor's blood supply. Although infused with a cold chemical preservative solution and chilled during transport, organs will fail to function if too much time passes before transplantation. This window is viability is known as "medically acceptable cold ischemic time." According to the United Network for Organ Sharing, 3,216 recovered organs went unused last year, several hundred of these because they could not be matched or transported to a suitable recipient in time.

Medically Acceptable Cold Ischemic Times:

Heart:	4 hours
Lung:	6 to 8 hours
Liver:	12 hours
Pancreas:	17 hours
Kidney:	24 hours

GRAPH: LETHAL MIDDLE GROUND: NORMAL OXYGEN LEVELS promote efficient energy production and cell function in most organisms. The authors and other research groups have also found that conditions of extremely low oxygen {anoxia} can prompt cells to enter a protective state of suspended animation in which they all but cease producing or consuming energy. When oxygen levels are intermediate {hypoxic}, however, cells attempt to continue operating normally, yet inadequate oxygen supply makes their activities inefficient and potentially self-destructive. Thus, oxygen-deprived tissues may be rescued by restoring their normal oxygen levels and also perhaps by blocking any remaining oxygen available to them.

PHOTO (BLACK & WHITE): BRAIN TISSUE from arctic ground squirrels shows the protective effect of natural suspended animation. Three days after slender [0.5-millimeter] probes were inserted into the brains of hibernating and nonhibernating squirrels, the animals were euthanized and their wounds examined. In the hibernating animal's tissue, a tiny hole made by the probe remains, but no other damage or evidence of inflammation is visible. In the nonhibernating animal, considerable cell death around the original injury left a large hole surrounded by darkly stained immune cells.

PHOTO (COLOR): AIRTIGHT GLASS CHAMBER was used by Roth and his co-workers to administer nonlethal doses of hydrogen sulfide (H(sub 2]]S) gas to individual laboratory mice of the type shown here for as long as six hours. In the group's experiments, the degree and speed of reduction in the animals' core body temperature and metabolic rate correlated with the H_2S concentration in the chamber, supporting their hypothesis that H_2S can induce a suspended animation-like state similar to natural hibernation in a mammal that does not normally hibernate.

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By Mark B. Roth, Todd and Nystul

MARK B. ROTH and TODD NYSTUL investigated the cellular mechanisms and protective effects of suspended animation together while Nystul was a graduate student in Roth's laboratory at the Fred Hutchinson Cancer Research Center. Roth's work encompasses many basic cellular processes, such as how cells regulate their own size, the expression of their genes, and their functional specialization. Nystul earned his Ph.D. From the University of Washington in 2004 and is now a postdoctoral fellow at the
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Carnegie institution in Baltimore, where he studies stem cell regulation in the fruit fly Drosophilo. In addition to preserving donor organs or critically injured patients, Roth and Nystul think that understanding the mechanisms of suspended animation may shed light both on stem cells' ability to remain quiescent and on certain cancerous tumor cells that are resistant to radiation because they exist in a similar low-oxygen and low-energy state.

LIFE IN BALANCE

Earth's earliest unicellular life-forms began evolving some four billion gears ago in an atmosphere that was nearly devoid of oxygen but very likely to have been chock-full of sulfur-containing molecules, such as hydrogen sulfide {H_2S). These primordial organisms came to generate their own energy supply by using H_2S in much the same way that most modern life uses oxygen. Indeed, many essential components of the oxidative phosphorylation pathway appear to have evolved from this earlier sulfur-based respiration mechanism Cytochrome c oxidase, for example, the component in the oxidative posphorylation machinery that normally binds oxygen, closely resembles the analogous component in sulfur-based respiration, and it can bind to hydrogen sulfide.

Oxygen metabolism and sulfur metabolism may share more than a simple ancestral relation, he Never. Even today H_2S is produced naturally by our bodies, which might seem incongruous given that H_2 binding to cytochrome c oxidase would inhibit oxygen's ability to do so. Yet it is possible that as ancient organisms started to make the transition to oxygen respiration, hydrogen sulfide took on a new role as an essential antagonist to oxygen.

The two molecules are highly reactive with one another and a constant give-and-take of electrons is fundamental to all life: some atoms give up their electrons in a process known as oxidation, whereas others take on electrons by "reducing" some other molecule's supply. These reduction-oxidation, or "redox," processes underlie energy production in all biological systems, and many organisms seek an environment where the potential for reactions is maximized.

In calm ocean water, for example, where dissolved gases mix primarily by diffusion, oxygen produced by photosynthetic organisms near the surface penetrates downward during the day and recedes at night, whereas H_2S constantly diffuses from below, an end product of metabolism by organisms that live off decaying material on the seafloor. The constant battle between these two gases creates a chemically unstable vertex where electrons are swapped at an alarming rate. This gradient is exactly the location that a host of organisms, such as the motile filamentous bacterium Beggiotoa alba, as well as many unicellular eukaryotes, choose to inhabit. These creatures' density can become so great that they form vast mats, which rise and fall in depth with the daily oxygen/H_2S cycle.

Perhaps our bodies and those of other oxygen-breathing organisms are like microbial mats seeking redox equilibrium. We do not live next to a source of H_2S, however, so we make our own, enabling our cells to remain in the optimal chemically unstable environment from which we evolved. I speculate that hydrogen sulfide's ability to bind to cytochrome c oxidase may have caused it to become part of an intrinsic cellular program to naturally slow or stop oxidative phosphorglation in the presence of oxygen. This protective mechanism would be useful at those times when cells risk harming themselves by struggling produce and use energy under anoxic conditions or, in the opposite situation, when an overdose of oxygen would cause cellular generators to overwork and potentially "fry" the cells. If H_2 were such a natural trigger for protective biological arrest, our success in employing it to induce hibernationlike states on demand would be explained. -M.B.R.

PHOTO (COLOR): LECHUGUILLA CAVE in New Mexico is one of many enclaves, such as deep-sea volcanic vents, where sulfur-oxidizing bacteria that probably resemble Earth's primordial life still thrive.

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