Volume 8 Spring 2003

MURJ Journal

Massachusetts Institute of Technology Undergraduate Research Journal

Massachusetts Institute of Technology

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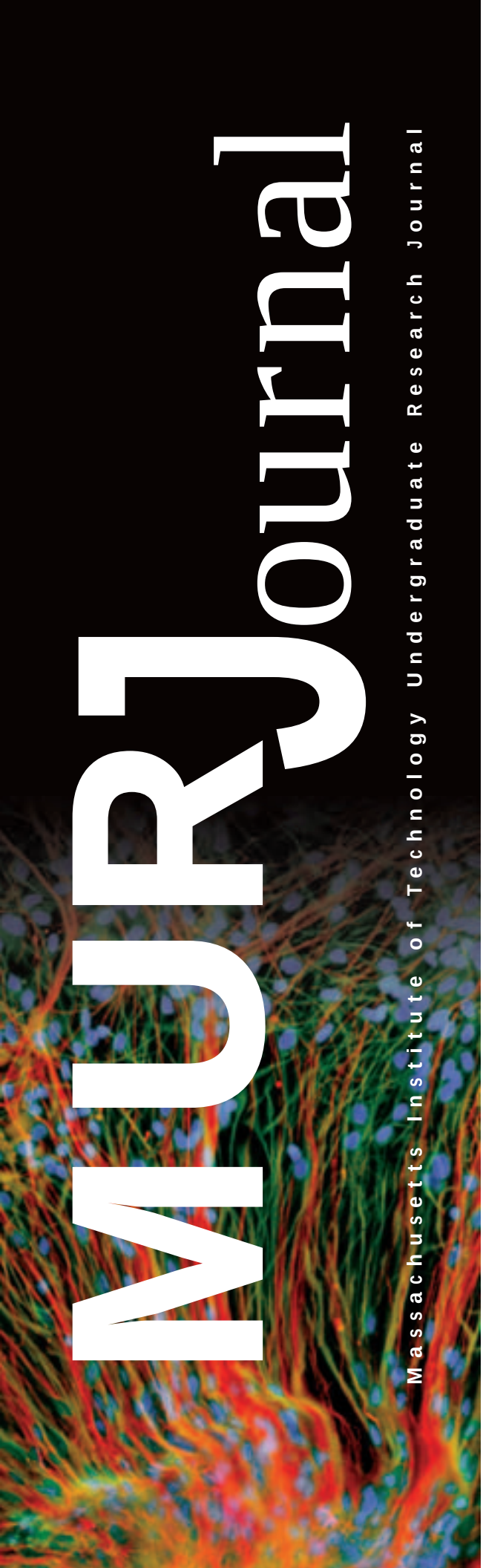

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Massachusetts Institute of Technology

UNDERGRADUATE RESEARCH JOURNAL

Volume 8, Spring 2003

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Massachusetts Institute of Technology
77 Massachusetts Avenue
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Welcome to the Spring 2003 issue of the *MIT Undergraduate Research Journal*. In this eighth issue of *MURJ*, we present diverse research reports in fields ranging from biological engineering to environmental accounting, in hopes that our readers will delve into the world of the scientific unknown. Within these pages we also include features examining topics such as stem cell research, cloning, factory health services, and scientific misconduct.

Our reports and features, as always, are written in such a manner as to make them accessible to all members of the MIT community. We hope that this interdisciplinary journal will provide an opportunity for our readers to learn of research in disciplines other than their own. We also hope that this journal will serve as a forum for debate and discovery through which our readers may explore arguments relating to the intersection of scientific research and public policy.

We extend our profound gratitude to those who have made this goal possible. We acknowledge the assistance of our advisor, Dean Les Perelman, the professors who reviewed our work, and the continued support of *The Tech*. We would also like to thank Dean Larry Benedict, the many academic departments at MIT, and the UROP and RLSLP offices for their financial support of *MURJ* this semester.

If what you read piques your interest, we invite your input for the Fall 2003 issue. Consider either joining the *MURJ* team or to submitting reports on your own research. Submissions will be due by the middle of September 2003. Please e-mail murj-public@mit.edu or visit our Web site, http://web.mit.edu/murj/www, if you have any questions or comments.

Sincerely,

The Editors of *MURJ*

MIT Science News in Review

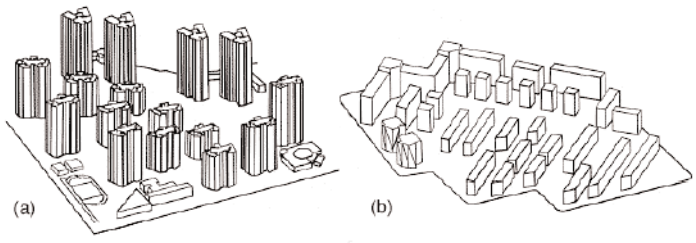
[Architecture]

Building Energy-Efficient Homes Brings Together Experts from MIT and China

Researchers from MIT's Building Technology Program and Chinese universities are working with developing companies to construct more comfortable yet energy-efficient living spaces for the Chinese population. The booming economy and the energy consumption in China have increased people's demand for more Western luxuries. Energy-efficient buildings have thus become the center of attention. The researchers have demonstrated that a traditional Chinese approach with additional building techniques can significantly improve the existing modern buildings. Although the recently erected buildings in China are mostly Western-style high-rises, the researchers have shown that low-rise buildings allowing more access to communal green space can also be energy-efficient. However, insufficient financial resources and a lack of workers who are familiar with the new technology have presented major setbacks. Lack of incentive to conserve energy also exacerbates the energy crisis. But the researchers on both sides are continuing their work to develop energy-efficient designs and to make the technology accessible to the Chinese builders.

In the meantime, demonstrations of the designs and the technology studies are being prepared in three major Chinese cities. Among the researchers are Leon R. Glicksman, Director of the Building Technology Program and a professor of building technology and mechanical engineering.

—W. Lee

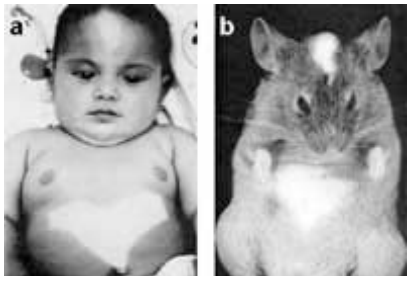


Airflow analysis is an effective technique for guiding the design of new communities in China. Scheme (a) is a design prepared by a Chinese architectural firm. Airflow analysis performed by MIT researchers showed that scheme (b) could provide the same living space while better blocking winter winds from the north, increasing passive solar heating in winter and enhancing cross ventilation from southerly winds in summer.

[Biological Sciences]

Of Mice and Men

The International Mouse Genome Sequencing Consortium recently produced a high-quality, publicly available draft of the mouse genome. An effort manned by scientists at twenty-seven institutions in six countries, including the Whitehead Institute/MIT Center for Genome Research, produced the genome in less than two years. This draft can now be compared with the recently sequenced human genome to gain insight about the functioning of genes, likely improving the understanding and treatment of diseases.



Already, interesting comparisons have been drawn between the mouse and human genome. Ninety percent of the mouse genome was found to have a corresponding region in the human genome. Only 5 percent, however, had exact groups of DNA bases that matched with the human genome. This 5 percent is thought to represent important functions such as protein-coding genes.

The mouse genome was determined to have fewer repeated sequences of DNA than the human genome, making it 14 percent shorter in length. These repeated sequences are not considered significant; they indicate that the mice have less extraneous DNA than humans do. Scientists also found a similar number of protein-coding genes to that of humans: approximately 30,000.

The sequencing of the mouse opens many doors for further genetic research. The mouse can now be utilized as a model for the study of a wide range of diseases. The new sequencing will also allow scientists to study the relationships between genes rather than only looking at each gene independently.

The consortium now plans to produce a final, nearly 100-percent-accurate sequence of the mouse genome. Other organisms such as the chimpanzee, cow, and honey bee will then be sequenced for further comparison between the genomes of different species.

—K. Rivoire

High-Carbohydrate Dietary Supplement Helps with Weight Loss

MIT researchers recently reported that a high-carbohydrate dietary supplement can help people who experience weight gain while taking antidepressants. The supplement includes a high-carbohydrate drink developed at MIT.

Even though the supplement altered serotonin levels in the body, there was no change in effectiveness of the antidepressant. The supplement also had a positive effect on nonmedicated obese individuals: During the twelve-week study, every participant lost twelve to twenty-six pounds.

People who take antidepressant medication, which increases serotonin activity level in the brain, usually experience weight gain by overeating sweet and starchy foods. The antidepressants block a serotonin receptor that also regulates protein and carbohydrate intake, which results in feelings of satiation. Because the receptors are involved in the antidepressants' therapeutic effect, the antidepressants usually cause weight gain.

By giving participants a carbohydrate-rich beverage twice a day, the researchers wanted to see whether increasing serotonin in the brain could reverse the obesity caused by antidepressants without affecting its therapeutic effects.

They found that obese individuals who had taken psychotropic drugs, such as antidepressants, were able to lose just as much weight as nonmedicated obese individuals. The treatment successfully increased serotonin levels without diminishing the therapeutic effects of the drug.

Co-author Judith J. Wurtman, visiting scientist at MIT's Clinical Research Center (CRC), examined the role of carbohydrates in the brain and their role in weight loss. She showed that carbohydrate craving is associated with serotonin-linked changes in mood and that woman with premenstrual syndrome (PMS) sometimes overeat carbohydrates and gain weight. She also thought that overeating increases brain serotonin, which diminishes feelings of depression and anger.

The study, supported by a grant from the Center for Brain Sciences and Metabolism Charitable Trust, was conducted at the TRIAD Weight Management Center at McLean Hospital in Belmont, Mass. —M. Kwan

New Breast Cancer Treatment Enters Final Stage of Clinical Trials

The U.S. Food and Drug Administration (FDA) has given MIT researchers approval to begin the final stage of clinical trials for testing an innovative breast cancer treatment using microwave radiation. The randomized clinical trials include the participation of nearly 220 women with early-stage breast cancer and began in October 2002, the first day of Breast Cancer Awareness Month.

The technology is based on radar research invented by Dr. Alan J. Fenn, a senior staff member at the Sensor Systems Division of the MIT Lincoln Laboratory. Fenn determined that the focused microwave technology previously studied for missile detection could possibly treat cancer cells. The clinical trials, based on Fenn's research, focus microwave radiation externally on the breast, heating and killing internal tumor cells, prior to lumpectomy and radiation therapy.

The randomized clinical testing is expected to finish by February 2004 and will be conducted at various hospitals including Columbia Hospital at the University of Oklahoma (OU), Harbor-UCLA Medical Center at the Martin Luther University in Halle, Germany, and the Comprehensive Breast Center in Coral Springs, Fla. Additional sites have applied for Institutional Review Board approval. Past studies of microwave heat therapy have been promising. Early results from a previous phase II clinical trial showed significant tumor cell death in a majority of the patients prior to lumpectomy, which led to the FDA's approval to begin the final phase of clinical testing.

Dr. Robert A. Gardner, a breast surgeon at Columbia Hospital's Center for Breast Care in West Palm Beach, Fla., and Dr. Hernan I. Vargas of Harbor-UCLA Medical Center presented the results of the phase II clinical trials at the 2002 American Society of Breast Surgeons meeting in April and in the May 2002 issue of the *Annals of Surgical Oncology*. The study is funded and led by Celsion Corporation, which has developed the clinical thermotherapy system and exclusively licenses the focused microwave thermotherapy technology from MIT. —C. Sadegh

Links: http://web.mit.edu/newsoffice/nr/2002/cancer.html
http://web.mit.edu/newsoffice/tt/2002/may08/breastcancer.html

Engineering Adult Stem Cells

Dr. James L. Sherley, principal investigator at the MIT Biotechnology Process Engineering Center, has engineered cells that behave like adult stem cells. Because adult stem cells are capable of generating new tissue, scientists hope to use them for organ replacement for their ability to develop into skin, red blood cells, and neurons.

Adult stem cells are unique in that they have immortal DNA; they pass on duplicates of their original DNA and thus avoid replication errors. However, these cells are extremely difficult to find because they appear in the body as normal cells.

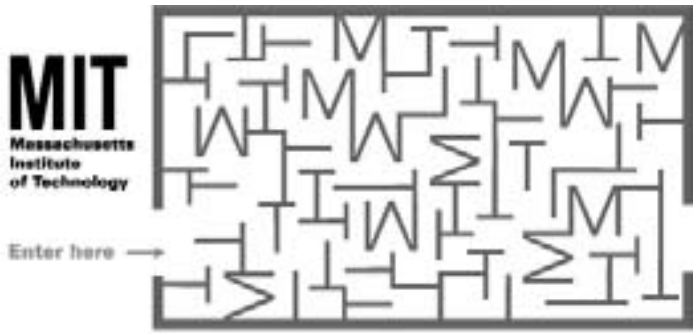
The cells that Dr. Sherley created have the capability to divide like adult stem cells given a certain culture

condition or to divide like cancer cells when treated in a different environment. The cells that he made can reveal biological mechanisms that answer questions about cancer and aging. The next step is to understand the proteins involved in stem cell division. —L. Giam

[Brain/Cognitive Sciences]

Slow-Wave Sleep Dreams in Rats Found to Replay Waking Tasks

MIT researchers reported in the December 19 issue of *Neuron* that rats dream about their waking activities during slow-wave sleep, not just REM sleep. The sleep cycle both in rats and humans is composed mostly of slow-wave sleep followed by sleep characterized by rapid eye movements (REM).



This study was a follow-up to a landmark 2001 study, also conducted by Associate Professor of Brain and Cognitive Sciences Matthew A. Wilson in MIT's Picower Center for Learning and Memory, showing that animals have complex dreams and are able to retain and recall long sequences of events, particularly during REM sleep.

In this study, Wilson and his co-author, graduate student Albert K. Lee, monitored the firing activity of collections of neurons in the rat's hippocampus as the rat ran on a simple track for a food reward. For each lap, different individual cells fired at different times, with each successive lap producing the same sequence of firings. Subsequently, in the slow-wave sleep directly following the activity, the rats were found to replay in short, high-speed bursts brief memory sequences corresponding to single laps. A 4-second lap on the track would only last 100–200 milliseconds in slow-wave sleep. This contrasts REM replay, which is played back in real-time.

Another difference was that the dreams found during slow-wave sleep only seemed to occur in the period of sleep immediately following the behavior. REM dreams that reactivate tasks, on the other hand, are detectable as long as 24 hours after the activity was performed, suggesting a more gradual reevaluation of older memories. Contrastingly, researchers have postulated that slow-wave sleep replay is part of the initial storage of memory processing during sleep.

Additionally, researchers found that the rats replayed only the stretches of running or attentive behavior, not the inactive periods of waiting in between. Thus, memory may not be as continuous as we tend to think it is. The authors have suggested that this study will be useful to create a model of how long-term memories are formed. "This may relate to work in humans that suggests that amount of slow-wave sleep early in the night, as well as the amount of REM sleep later in the night, is correlated with subsequent enhancement of performance on learned tasks," said Wilson. —S. Brenner

[Electrical Engineering and Computer Sciences]

Virtual Touch of Loved Ones Now a Reality

On May 23, 2002, MIT and University College London researchers collaborated on the first touch exchange across the Atlantic. The MIT team led by Mandayam A. Srinivasan worked with software specialists at UCL to transform a commercially available, MIT-developed robotic arm, the PHANToM, into a haptic (touch) machine.

UCL researchers worked on the software that runs the robotic arm and the networking communication involved in the transatlantic experiment, while MIT engineers worked on adapting the PHANToM into a machine that can translate touch by exerting exact pressure onto the operator's fingers.

In the May experiment, the two participants on both continents were simultaneously placed in a virtual room on a computer. In the room was a box and pointers that represented the participant's location in the room. The participants then tried to work together to raise the box; each participant's individual motion affected the box's position and could be felt by the other.

But the experiment didn't run perfectly. Due to network delay and Internet traffic there was a delay in the transmission of the real-time touch. The delay was only around 150–200 milliseconds, but compared to a signal sent from the hand to the brain, which takes only 30 milliseconds, this delay is a major obstacle. As a result, the arm operators had a hard time keeping in sync with each other.

The next step for the UCL and MIT collaboration is to minimize the traffic delay to less than 30 milliseconds and to develop a better touch algorithm for the promising machine. Just think, one day surgeons could operate on patients thousands of miles away, and 8.02 students could feel the intranuclear forces inside atoms as part of their lab.

—T. He

Ultra-Thin Quantum-Dot LED Raises Prospects of Better Flat-Panel Displays

MIT researchers have developed a quantum-dot-organic, light-emitting device (QD-OLED) using a novel combination of thin organic materials and high-performing inorganic nanocrystals. The research raises the possibility of manufacturing these materials to create thinner and brighter flat-panel electronic screens to replace today's popular liquid crystal displays (LCDs).

Quantum dots, or artificial atoms, are nanometer-scale boxes that selectively hold or release electrons. While LCDs must be lit from behind, quantum dots emit their own light. Depending on their size, the dots can be "tuned" to emit any color in the rainbow as well as in the infrared and ultraviolet ranges. And the colors of light they produce are much more saturated than that of other sources. The QD-OLEDs created in the MIT study also have a twenty-fivefold improvement in luminescent power efficiency over previous QD-OLEDs.

This latest MIT QD-OLED contains only a single layer of quantum dots sandwiched between two thin, organic films, whereas previous QD-OLEDs used ten to twenty layers. The researchers have demonstrated organized assemblies larger than 1-square centimeter, and the same principle could be used to make bigger components.

Moungi G. Bawendi, professor of chemistry, and Vladimir Bulovic, assistant professor of electrical engineering and computer science, led the interdisciplinary research on the hybrid optoelectronic structure for the QD-LED. The collaborative effort is supported by MIT's Center for Materials Science and Engineering (CMSE). Bulovic is also affiliated with the Research Laboratory of Electronics.

In addition to being used for extraordinarily thin and bright flat-panel displays, the QD-OLEDs may also be used in a variety of other applications: to calibrate wavelengths for scientific purposes, to generate wavelengths visible only to robot eyes, or for the miniaturization of scientific equipment. The MIT team's method of combining organic and inorganic materials may pave the way for new technologies and enhance understanding of the physics of these materials. Further understanding of the material properties of QD-OLEDs will open doors for the production of flat-panel displays that are stable, simple to produce, high-resolution, and highly efficient.

The work, reported in the December 19 issue of *Nature*, is funded by the National Science Foundation's Materials Research Science and Engineering Center Program and Universal Display Corp.

—C. Sadegh

[Materials Sciences]

Lithium Ion Phosphate Batteries Take Charge

In a major breakthrough published late last year in *Nature Materials* magazine, MIT Department of Materials Science and Engineering (DMSE) researchers claimed to be one step closer to making inexpensive, safe, and rechargeable batteries a reality. The researchers proposed using cells made of a new lithium ion phosphate–based material, which is seen as the possible next generation for energy cells.

For years, industries have been trying to find a new, safer, and cheaper rechargeable material for batteries that is less chemically reactive than the current lithium-cobalt-oxygen cells. Such cells can overheat easily, causing producers to limit the size of such cells.

Researchers at the University of Texas-Austin first suggested looking into lithium ion phosphate as a potential substitute. Although cheap and environmentally safe, however, the chemical has a very low electrical conductivity so at the time it was impractical as a rechargeable cell.

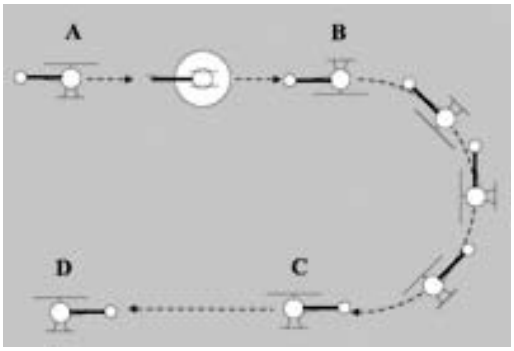
Professor Yet-ming Chiang led the MIT research team that added some metals and developed a special process for treating the compound, which increased its conductivity ten-millionfold! As a result, the lithium ion phosphate battery is quickly becoming a reality. Impending tests will allow scientists to scrutinize and verify the results, so expect to see lithium ion phosphate batteries take charge of the rechargeable battery market.

—T. He

[Mechanical Engineering]

Helicopter Maneuvers Autonomously

MIT researchers developed a pilot model X-Cell 60 helicopter, which executed a complicated maneuver autonomously: The split-S maneuver allowed it to reverse its direction in a limited amount of space.



In the past, complicated maneuvers required a skilled pilot, but now these advances will be applied to unmanned aircraft for use in national defense. The autonomous helicopters can be used for collecting military intelligence and imagery when it is unsafe for other aircraft to enter the airzone. These autonomous aircrafts are small, inexpensive, and easy to control, which make them suitable for military operations.

— L. Giam

[Physics]

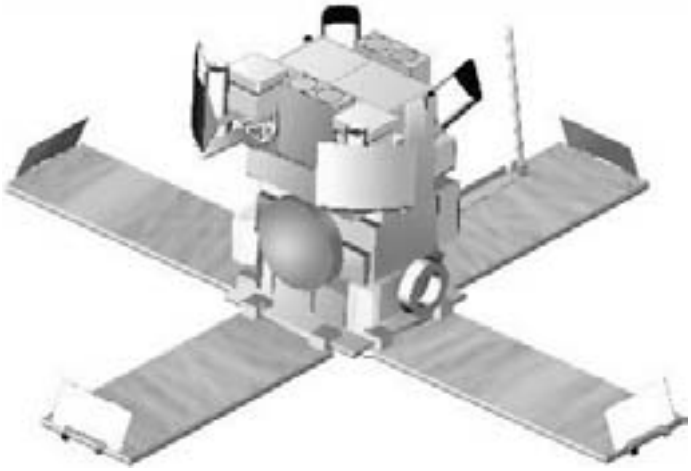
Nuclear Fusion Envisaged as Major Source of Energy in Future

Nuclear fusion involves the joining of lighter elements under high pressure to produce heavier elements as well as tremendous amounts of energy. Magnets dictate the behavior of the plasma in which the fusion takes place. Scientists at MIT's Plasma Science and Fusion Center and the Department of Nuclear Engineering have created a cylindrical, 150-ton magnet for the International Thermonuclear Experimental Reactor (ITER). This magnet will form part of a bigger magnet weighing 925 tons, which in turn will be part of a total magnet system weighing some 10,000 tons. In recent years, a number of tests have been conducted on the 150-ton magnet, located in Japan. This magnet can produce a magnetic field of 13 Tesla and can store energy of 640 megajoules at a current of 46,000 amperes; for reference, the current handled by typical household wiring is around 20 amperes. It was also found that the magnet can be operated in pulses, and it can be brought up to 13 Tesla and back down in only a few seconds. Suitable ranges for the magnetic field, temperature, and current density continue to be defined as support for the initiative. This past September, a Department of Energy panel recommended that the United States rejoin the ITER collaboration along with Japan, Germany, and Russia. The U.S. team involved in developing ITER is composed of about 20 researchers from the MIT Plasma Science and Fusion Center, and the project is funded primarily through a multiyear grant from the Department of Energy to MIT.

— M. Sircar

First Rapid Detection Sheds New Light on "Dark" Gamma-Ray Bursts

The first X-ray image of a rare fast-fading "dark" gamma-ray burst was taken by the MIT-built High Energy Transient Explorer (HETE) satellite, the first satellite dedicated to spotting gamma-ray bursts.



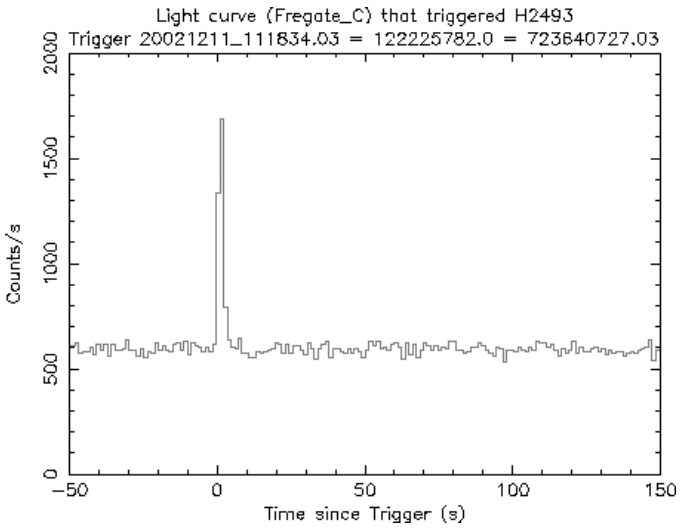
A signal for the birth of a new black hole, a gamma-ray burst is the most powerful type of explosion known, second only to the Big Bang in total energy release. "Dark" gamma-ray bursts are aptly named because prior to the recent X-ray image, they have had no detectable optical afterglow. Other bursts have bright afterglows that linger for days or weeks, likely caused by the explosion's shock waves ramming into and heating gas in the interstellar medium.

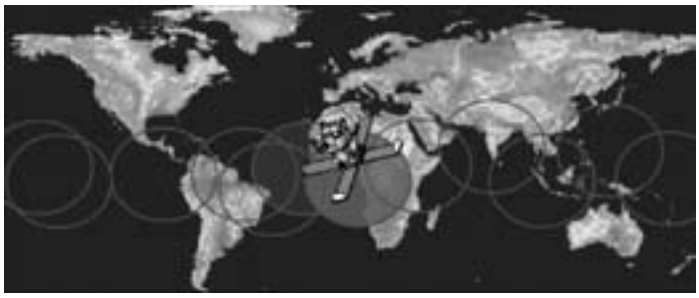
The orbiting HETE, which alerts scientists to gamma-ray bursts, spotted one on December 11, 2002, originating six billion light years away, and relayed its location to

observatories worldwide in 22 seconds. The ground-based Raptor optical telescope, operated by the Los Alamos National Laboratory, was the first on the scene, observing the afterglow at 65 seconds. The afterglow was extremely faint after two hours and would have been missed and labeled "dark" if not for HETE's rapid detection.

"Perhaps none of these bursts is truly dark, provided that we catch them fast enough," said George Ricker, a senior research scientist at MIT's Center for Space Research, who leads the international team that built and operates NASA's HETE satellite. Some theorists have suggested that dark bursts have no detectable afterglow because they are buried in thick dust and gas, which blocks the afterglow's light from reaching Earth.

According to Ricker, the December 11 observation of burst implies the opposite: "The burst may have occurred in a region with hardly any surrounding gas and dust, thus the shock waves had little material to smash into to create





a prolonged bright afterglow." The rapidly fading afterglow, in this case, may support the binary merger theory of short bursts. In the billions of years that old binary systems, with a combination of neutron stars or black holes, took to form, they drifted outward to less dense regions of a galaxy. Thus, when they merge to form a black hole, there is little surrounding material to make a long afterglow.

HETE was built by MIT as a mission of opportunity under the NASA Explorer Program. It is on an extended mission until 2004. The HETE program is a collaboration between MIT; NASA; Los Alamos National Laboratory, New Mexico; France's Centre National d'Etudes Spatiales (CNES), Centre d'Etude Spatiale des Rayonnements (CESR), and Ecole Nationale Superieure del'Aeronautique et de l'Espace (Sup'Aero); and Japan's Institute of Physical and Chemical Research (RIKEN). The science team includes researchers from the University of California (Berkeley and Santa Cruz) and the University of Chicago, as well as from Brazil, India, and Italy. —C. Sadegh

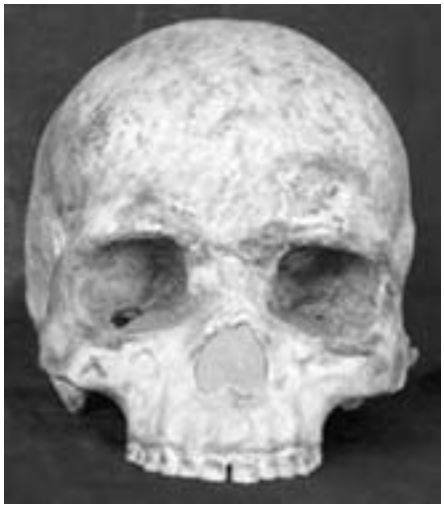
For more information: HETE: http://space.mit.edu/HETE

Additional images and GRB021004 information:
http://space.mit.edu/HETE/Bursts/GRB021211
http://web.mit.edu/newsoffice/nr/2002/darkburst.html

World Science News in Review

[Archaeology]

Fossils Found in China Could Be Earliest in East Asia



In 1958, in the county of Liujiang in southern China, farm workers discovered several human bones, including a skull, while digging in a cave. Because it resembles to-date fossils in Japan, scientists estimate this *Homo sapiens* skull to be from 20,000 to 30,000 years old. However, according to an article in the December 2002 J*ournal of Human Evolution*, the fossils might be much older than that.

The fossils probably came from sediment dating to 110,000 to 139,000 years ago, estimates a team led by geologist Guanjun Shen of Nanjing (China) Normal University. He also said the fossil discoveries probably came from either the cave deposit dating from 68,000 years ago or one dating to more than 153,000 years ago.

The team concluded that if any of the estimates are true, "the Liujiang [specimen] is revealed as one of the earliest modern humans in East Asia." This would roughly coincide with the earliest known fossil dates in Africa and the Middle East.

Yet evidence of the skull may complicate the human-origins debate, creating problems for the out-of-Africa theory of human evolution—that modern humanity started in Africa between 100,000 and 200,000 years ago and then spread elsewhere. If the fossils found in Liujiang are dated accurately, supporters of the out-of-Africa theory will have to find even older African *Homo sapiens* fossils. Another possibility for the adherents would be showing that modern humans migrated very quickly from Africa to Eastern Asia.

The scientific accounts from 1959 and 1965 of the fossil discoveries led to the new determination of the likely burial site of the fossils. Shen and his coworkers mapped various soil deposits in the cave and used uranium decay methods to work out the age of the crystallized limestone. According to Shen, other sites' uranium samples have been analyzed and support the idea of ancient human origin in southern China. Teeth that were discovered in two other caves have been estimated to be at least 94,000 years old, said Shen.

On the other hand, some anthropologists doubt the estimated age of the Liujiang skull. It's still uncertain how the skull got in the cave and where it was originally buried, remarked Christopher B. Stringer of the Natural History Museum in London.

Milford H. Wolpoff of the University of Michigan in Ann Arbor agreed. "I'd love for the Liujiang skull to be as old as Shen proposes, but we'll never know for sure without directly dating the specimen."

Shen said he hopes he can get an agreement with the Chinese officials to date the specimen directly. —M. Kwan

[Biological Sciences]

Nervous System Drug Shows Promise for Male "Pill"

Researchers have discovered a new oral drug, known as N-butyldeoxynojirimycin or NB-DNJ, that prevents sperm cells from developing normally in mice. Until now, the only FDA-approved male birth control methods have been condom use and vasectomy, though a practical birth control pill for men has long been sought after.

Researchers at the University of Oxford in England stumbled upon NB-DNJ's potential birth control abilities while exploring its use in treating diseases of the nervous system, during which male mice exposed to the drug

became temporarily infertile. "So, we started a study to look at what this drug was doing to male-mouse fertility," said Frances M. Platt.

She and her colleagues at Oxford spiked the mouse feed with varying doses of NB-DNJ and found that the females coupled with the control males produced normal litters, but the treated males, even those who were given low doses of the drug, could not impregnate the females.

After further investigation, the researchers found that NB-DNJ damages the sperm mitochondria and nuclei, thus impairing the sperm's swimming ability. The drug also prevents the formation of a cap, called the acrosome, on the sperm's head, which normally enables the cell to penetrate an egg.

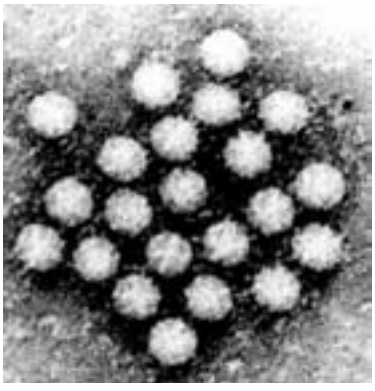
The effects of the drug are reversible: Sperm of the mice treated with any dose of the drug for up to six months (which is a significant portion of the average two-year lifespan of a mouse) were able to regain fertility by three weeks after researchers stopped administering the drug.

Though a version of NB-DNJ has been approved by the European Union for the treatment of Gaucher's disease, the drug's use as a male birth control pill will require further scrutiny of its known toxic effects, such as those affecting sperm internal parts.

NB-DNJ appears to be less risky than some other drugs being studied for their male birth control potential, stated Ronald Swerdloff of the Harbor-UCLA Research and Education Institute in Torrance, Cal. He added that NB-DNJ "is a long way from a final, tested product, but it seems to be an exciting lead." —F. Merali

Noroviruses Cause Many Shipboard Outbreaks

According to recent reports from the Centers for Disease Control and Prevention (CDC) in Atlanta, cruise ships' sanitation practices do not reliably wipe out viruses that cause diarrhea outbreaks. Although cruises are cleaner today than in past years, stopping the viral scourge on ships will require even more rigorous measures, including completely scrubbing down affected vessels.



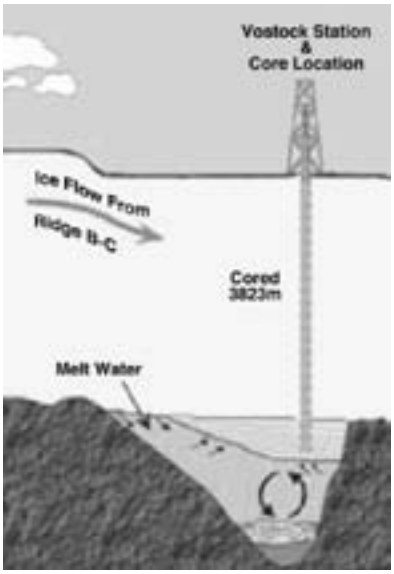
The viruses, called Noroviruses, pervade easily through casual contact and can last for many days in the open air. The viruses often set off epidemics of diarrhea and extreme vomiting in cramped spaces on ships, military camps, and overcrowded areas. They also cause approximately 23 million illnesses in the United States every year.

In 2002, at least 23 outbreaks occurred on cruise ships, in which more than 2,000 people were sick on five ships operated by four different companies.

On three of the five ships, these outbreaks affected several consecutive voyages because the virus probably survived onboard between scheduled cruises. The preventative measures used on the voyages ranged from scrubbing public areas to sanitizing poker chips. In one particular case, the virus struck Holland America's *Amsterdam* on four different voyages. The outbreak started to subside only when the ship was docked for ten days and underwent intense cleaning.

In 2002, the wave of viral outbreaks contrasted from an overall downward trend in stomach infections on the ship. In the 1990s, inspectors reported improper handling of water on 55 percent of inspections and food on 62 percent. They also reported a 95 percent equipment maintenance and dishwashing violation rate. Despite these scores, half of all cruise lines received passing scores. —M. Kwan

Life under Thick Ice in Antarctica Lake

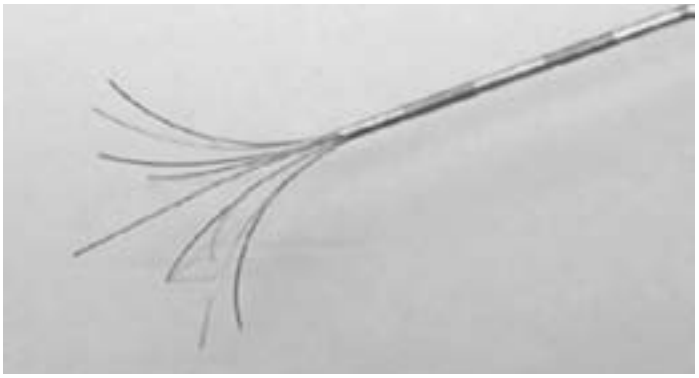


In 1996, the collaboration of Russian and British glaciologists helped discover Lake Vostok, one of the largest and deepest lakes, below 4,000 meters of ice in Antarctica. Three years later, the scientists were able to map out the dimensions of the territory with advanced technology. The biggest mystery that has bewildered all scientists is whether life exists in the lake. In 1999, curiosity drove the National Science Foundation to initiate an airborne survey of the lake and later an actual expedition to the lake at the Vostok Station. It is highly suspected that ancient microbes or life forms do dwell in the lake. Cylindrical ice samples have been pulled from the lake for examination, but the research was stopped in 1998 for fear of contamination. Yet from what has already been extracted, scientists have found a variety of living organisms—from bacteria to algae—three-fourths of the way down the lake, and current researchers are working to find viable microbes even deeper into the

lake. Although the physical conditions at this level make the chances of finding organisms slim, scientists expect to find microbe existence. The study of Lake Vostok and its possible inhabitants has also attracted NASA, which planned to use the lake as a practice site for an expedition to Europa. —W. Lee

Medical Procedure Eases Pain in Bone Cancer Patients

For most cancer patients, experiencing physical pain is perhaps one of the most difficult consequences of the disease. However, scientists at a recent meeting of the Radiological Society of North America (RSNA) announced a new pain-reducing treatment for bone cancer patients: radiofrequency ablation (RFA).



RF ablation electrode with full deployment.

Utilizing computer tomography (CT) and ultrasound guidance, RFA transmits high-frequency electrical currents through a needle to the targeted tissue, thus heating and destroying a large part of the tumor. Since the process successfully eliminates a large portion of the lesion, RFA substantially reduces the pain in patients. According to Dr. Matthew Callstrom of the Mayo Clinic in Rochester, Minnesota, RFA has been previously used to treat other cancers such as those in the lung and kidney tissue. The ablation procedure has recently been used to treat patients with metastatic bone cancer. Metastatic bone cancer results from the spreading of a malignant tumor via the bloodstream to one or more bones near the body's center, such as the hip or spine.

Callstrom, who works for one of the nine research centers in the United States and Europe participating in the study, confirmed that RFA has successfully eliminated pain in patients with metastatic bone disease. Approximately 81 percent of the patients attained "a high degree of pain relief," and nearly half experienced "complete elimination of pain" six months following treatment. Furthermore, the patients reported a decrease in physical pain from 7 to 2 on the standardized Brief Pain Inventory (BPI), a 10-point scale with 10 being the highest level of pain. Taking these results into account, Callstrom optimistically expressed that RFA offers a safe and effective treatment option to reduce severe pain so as to "improve the quality of the patients' lives."

However, what RFA can't do is successfully eliminate pain in patients whose cancer has spread throughout their entire skeletons—it can only help lesions. Despite this drawback, the outlook for this new treatment is optimistic: The U.S. Food and Drug Administration (FDA) officially approved the use of RFA for treatment in December 2002. —L. Peng

[Chemistry]

Findings of Acrylamide in Food May Lead to Health Risks

Those who love to eat French fries may be shocked to hear that what they are eating does not only contain high fat content, but also contains acrylamide, an agent that causes cancer in rats. According to the U.S. Food and Drug Administration (FDA) and Germany's Bavarian Ministry of Health, scientists have reported findings of acrylamide in foods like French fries and gingerbread.

Acrylamide, a chemical substance used in the production of plastics, is created by high-temperature cooking methods, especially in starches. The FDA has already initiated testing of acrylamide levels in starchy foods such as French fries and potato chips. The researchers first tested for acrylamide concentration in French fries by baking numerous batches of different types of fries, from various chain restaurants to various home-cooked (store-bought) types (see table). For restaurant-cooked fries, the acrylamide concentration ranged from 117 ppb (parts per billion) at KFC to 1030 ppb at Popeye's. For home-cooked fries, Ore Ida brand registered at 616 ppb and Lamb Weston Island Valley peaked at 1325 ppb.

Acrylamide Concentrations (ppb) in French fries at different locations of chain restaurants
(FDA preliminary data)*

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7
Burger King	197	220	369				
Checkers	257	407					
Fuddruckers	346	452					
KFC	117	162	270	313			
McDonald's	155	193	245	270	328	356	497
Popeye's	301	484	610	1030			
Wendy's	157	169	254	260	302		

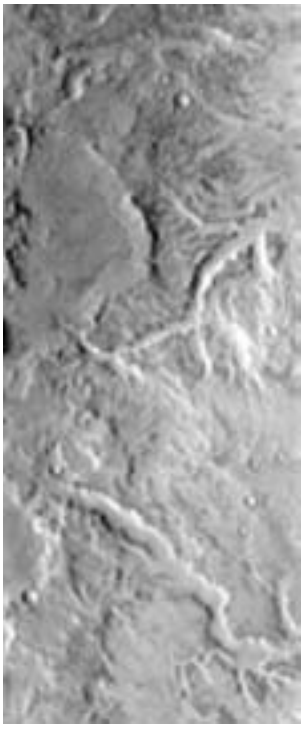
Other foods that tested positive for acylamide concentrations were fish fillets (30 ppb), Pepperidge Farm Cheddar Goldfish crackers (57 ppb), unbrewed Starbucks Coffee Columbia Ground (175 ppb), Hershey's Cocoa (909 ppb), and Lipton Soup & Dip Mix (1184 ppb).

What should one make of all these numbers? Even though these foods contain acrylamide, these results are only preliminary and do not currently pose a major health risk to Americans, according to the FDA. More research is needed to form a better conclusion about the potential health risks that acrylamide might pose. —L. Peng

Source: *Science News*, "Acrylamide—from Spuds to Gingerbread."

[Earth, Atmosphere, and Planetary Sciences]

Collisions Heat Up Mars Climate and Erosion Debate



A group of physics-driven planetary scientists propose that the mysterious erosion markings on Mars can be explained by giant asteroid collisions. The source of water for the massive amounts of erosion has remained a mystery for about thirty years. Climate models cannot explain why the frozen planet was warmer in its early years. However, a new physics-based approach is generating very convincing ideas and numbers. The group calculates that a relatively small asteroid measuring 100 kilometers delivered about 4 x 1,026 joules of energy to the planet.

The energy of the impact, combined with the deep layer of hot rock debris that covered the planet afterward, brought global surface temperatures to about 800 K. As a result, the frozen planet thawed on its surface, at its polar ice caps, and in its subsurface. A small 100-kilometer object impact would result in 5 meters of water covering the planet for at least one year. A single 250-kilometer asteroid might have melted 50 meters of water for at least a century. At least ten such objects collided with Mars, punctuating the planet's cold, dry climate with episodes of rain and erosion. According to the new research, the cumulative effect of the impacts can account for the massive erosion.

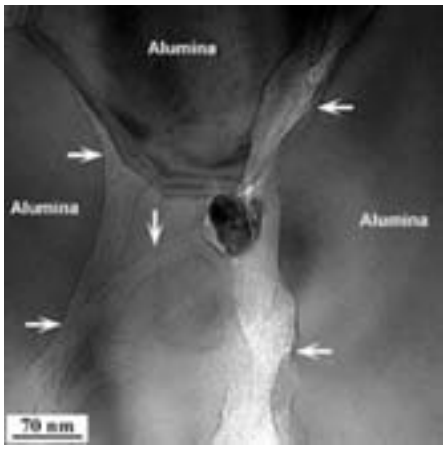
Geologists point out that this new theory may explain the earlier stages of erosion, but it cannot account for the later valley formations and flooding of craters. The amount of erosion seems to have required kilometers of water, much more than the 50 meters produced by an impact. To attract more supporters, the physics-driven planetary scientists need to develop a better model of the cumulative effects of impacts over time. —S. Kan

[Materials Science]

Size Doesn't Matter: Nanotubes Play Big Role in Strengthening Ceramics

University of California-Davis researchers, led by Joshua D. Kuntz and Amiya K. Mukherjee, have managed to triple the fracture resistance of ceramics. The scientists accomplished the feat by introducing small amounts of carbon nanotubes into the mix of alumina crystals to make a superstrong material.

Many researchers have tried to introduce carbon nanotubes as the agent to eliminate the infamous brittleness of ceramics. The UC-Davis researchers strayed from traditional approaches in two ways: the type of nanotubes used and the technique in hardening the ceramics. Previously, other researchers had relied on multi-walled rather than single-walled nanotubes. In addition to using



the different type carbon nanotubes, the UC-Davis scientists also employed a low-temperature heating technique to harden the ceramic. They suspected that the previous sintering technique that operated at a higher temperature damaged many of the nanotubes.

The two changes caused a two-and-a-half-fold increase in fracture resistance compared to the old method. (The previous best had only decreased the brittleness of the alumina crystal ceramics by 24 percent.)

With this significant increase in shatter resistance, the future for the new ceramic material is boundless; it could be used for gears, industrial parts, and the like. But until researchers can find a way to lower the price of the expensive carbon nanotubes, the material will probably be used only in heavy industries, where production cost is not the main concern. —T. He

[Mathematics]

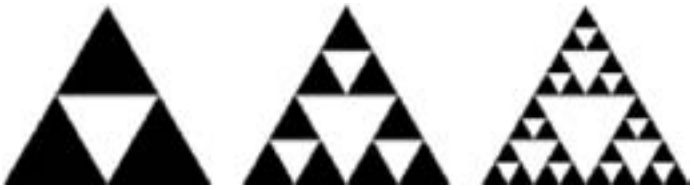
Mathematical Forays into Musical Compositions

In Swedish composer Daniel Cummerow's musical works, each musical fragment is determined by a mathematical recipe through the use of a formula that links numerical digits with musical notes. For example, the mathematical constant p, has an intricate, vaguely medieval correlate, whereas the decimal digits of the constant e, progress at a relentless, suspenseful pace.

A variety of different strategies are used by algorithmic music composers to get such interesting results. One method involves converting prime numbers directly into their corresponding MIDI notes, at least up to 127, to get a curiously rising scale. The MIDI specification assigns a number to each note on a keyboard (e.g., middle C is 60, C-sharp is 62, and so on, for a total of 128 tones) and is used in computer programs to represent musical notes. However, since there are an infinite number of primes, one could continue by dividing each prime by a certain number, then use just the remainder to assign the musical value—an elegant use of modular arithmetic.

In some of his pi compositions, instead of mapping digits directly to their respective MIDI numbers, Cummerow constructs the piece by assigning each digit from 1 to 8 to a note in a specific scale; 0 signals a pause; and 9 means either a pause or the repetition of the previous tone.

In yet another experiment, Cummerow uses a unique musical alphabet invented by French composer Olivier Messiaen, which extended the German names of the notes A to H by giving each letter of the alphabet its own pitch, octave, and note value. Cummerow paired the first 255



digits of pi and applied the above formula to those that fell below twenty-six. For the rest, he followed a different recipe.

Other techniques have yielded fascinating musical pieces featuring the Fibonacci sequence, Pascal's triangle, and intriguing structures that have been associated with chaotic dynamics, such as the Sierpinski triangle and Lorenz's butterfly.

Many other musicians have also delved into mathematical music, exploring ideas such as the fractal notion of self-similarity in which each component is a miniature replica of the overall structure.

Only rarely do composers enter the seemingly forbidding world of mathematicians and their abstruse concerns, though the recent frenzy of forays into the field may be just the start of a beautiful marriage between mathematics and music. —F. Merali

Intriguing mathematical music compositions of the following artists can be found on the World Wide Web: Cummerow (http://www.geocities.com/Vienna/9349/); Chris K. Caldwell (http://www.utm.edu/research/primes/programs/music/listen/); and José Oscar Marques (http://www.midiworld.com/c/jmarques.htm).

A Step Closer to Finishing Pi

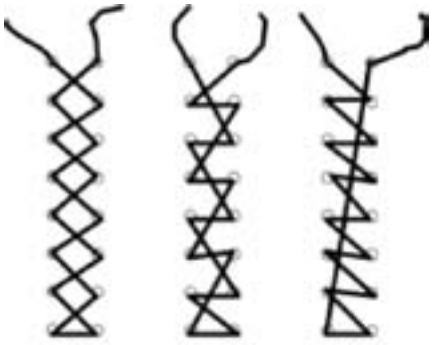
At the University of Tokyo Information Technology, Yasumasa Kanada and his colleagues have been able to calculate the value of p up to 1,241,100,000,000 digits, the last of which is 5. Thanks to the improvements in the algorithm to calculate π and 602 hours of a Hitachi SR8000 computer's time, the group was able to set this new record, surpassing their previous world record in 1999 of 206,158,430,000 digits. As expected, initial statistical analyses indicate that each of the digits from 0 to 9 appears with the same frequency. Questions about the distribution and apparent randomness of π's digits still remain: Do all digits appear infinitely often? At some point beyond the range of current computations of π's value, do its decimal digits revert to a string constrained of only digits of say 1 and 0 (or any other number(s))? —M. Sircar

Survival of the Fittest Shoelaces

Computer scientist John Halton and Australian mathematician Burkard Polster recently investigated a problem to which even a small child can relate: how best to lace one's shoes.

Though there exist more than 50,000 possible lacing methods for a shoe with as few as five eyelets per side, over time, humans have gravitated toward several common lacing patterns. The three most common lacings are criss-cross, also termed "American," European straight, and shoe store straight.

Through mathematical modeling of the shoelace as two parallel lines, each with n points, Halton and Polster were able to evaluate the strongest and shortest lacing patterns. Halton discovered that the zigzag American pattern required the least amount of lace for a given number of eyelets, with the European straight next, followed by shoe store straight, provided that at least four eyelets are present on each side of the shoe.

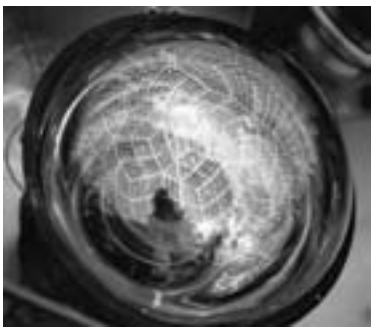


Polster investigated less traditional lacings as well, finding that a bow tie lacing in fact uses the least lace of all. When considering which lacing is strongest, however, Polster concluded that a "dense" lacing such as the American style that crosses back and forth between sides of the shoe is best.

—K. Rivoire

[Physics]

Antineutrino Transformations Detected on Earth



Recent evidence gathered by an international group using an underground detector known as the Kamioka Liquid Scintillator Antineutrino Detector, or KamLAND, in Toyama, Japan, indicates that electron antineutrinos morph into muon or tau antineutrinos on Earth, as they do on the sun. The research group determined this by monitoring light flashes caused by electron antineutrinos generated by a few dozen nuclear power reactors in Japan and Korea. KamLAND, set up in a mine one kilometer below ground level, contained thousands of tons of organic liquids that emitted light when struck by these electron antineutrinos. Because only 63 percent of the expected number of electron antineutrinos, and thus light flashes, were detected, physicists concluded that the electron antineutrinos had oscillated into the two other possible forms—muon and tau antineutrinos. These earthly findings closely follow observations of transformations of neutrinos and antineutrinos emitted by the sun. Giorgio Gratta of Stanford, the lead scientist of the study and a KamLAND spokesman, concluded that oscillations are "a property of neutrinos and not that of the sun."

—M. Sircar

Factory Health Services:

An Innovative Method of Providing Health Care in Bangladesh

Aadel Chaudhuri

Health care in Bangladesh is a complicated issue. Despite having a land area of merely 55,598 square miles, Bangladesh has a population of 133 million people, making it the eighth most populated country in the world and the twelfth most densely populated.[1,2] Additionally, Bangladesh is one of the world's twenty least-developed nations.[3] It comes as no surprise that due to the high population density, coupled with the country's low level of development, health care for the masses is a major concern that is rather difficult to address.[4,5] Funding agencies such as the United Nations Children's Fund (UNICEF), the United States Agency for International Development (USAID), and the International Center for Diarrheal Disease and Research in Bangladesh (ICDDRB) have recognized this dilemma and have thus helped Bangladesh's government and nongovernmental organizations (NGOs) provide health care to the country's great many people.[4,5]

The World Bank is one of many organizations dedicated to helping Bangladesh's health program.

Bangladesh's Government Health System

Despite the daunting challenges of providing health care, Bangladesh's government health system is very well-organized. There are hospitals and clinics for every societal class. Big hospitals cater to cities. More limited but still extensive clinics provide services to *upazilas*, which are composed of a few districts. Small clinics cater to individual districts. Finally, small doctor rooms or a couple of doctors take care of individual unions or villages. The government provides its health services at very low costs, with the ultimate goal of providing free health care to all. Thus, the government attempts to target all members of the population, even the poorest residents of the remotest villages.[3]

A Bangladeshi Slum Area.

A cook at work.

Unfortunately, although planned well, the government system has a number of drawbacks, one of which is inefficiency. There are simply too few doctors available to see the patients that come in. This often forces patients to wait exorbitant amounts of time before being able to see a doctor.[6] Additionally, funds are often insufficient or misappropriated, which results in half-constructed operation theaters or unmanned examination rooms in hospitals and clinics.[6] As a result of the government's apparent inefficiency in the health sector, only 30 percent of Bangladesh's population utilizes the government's health services.[7]

A further disability of the government's system is that it caters too specifically to rural areas. The structured hospital/clinic organization previously detailed seems mostly to be designed with rural areas in mind.[7,8] The lack of "remote areas" in large, dense cities such as Dhaka makes a tier system seem almost unnecessary because the location of the large city hospital may be just as strategic as the site of the upazila health clinic, with both areas being equally densely populated and publicly accessible. In short, the government's health system has not been designed to specifically serve urban areas, where the patient need is greatest.

Involvement of Nongovernmental Organizations

Although the Bangladesh government health system's weaknesses are unfortunate, NGOs have heroically stepped in to help supplement the ailing system. NGOs have set up clinics in both urban and rural areas and have helped provide low-cost health care to the poor and the very poor.[9,10] The role of NGOs is especially apparent in urban areas, where the government's system is not able to sufficiently serve the large population.[6,8,9] Additionally, NGOs are committed to teaching health awareness and providing health counseling.[9,11,12] They have successfully shown that they can improve people's health by suggesting lifestyle changes.[9,10,11] The government does not provide and promote such health awareness-related services as readily as the NGOs do.[6,7,9] Thus, the NGOs have effectively filled in many gaps of the government's health system, supplementing and supporting it so as to provide more people with necessary health care.

Mary Stopes Factory Health Services

Mary Stopes Clinical Society (MSCS), a Bangladeshi NGO that is affiliated with Mary Stopes International (based in the United Kingdom), has established clinics and mini clinics in Bangladesh's major cities, focusing on reproductive health but also covering general health services.[9,11,12] Other NGOs, such as the Bangladesh Rural Advancement Committee (BRAC) and the Bangladesh Association for Maternal and Neonatal Health (BAMANEH), are acting similarly as well as reaching into rural areas.[10] Mary Stopes, however, is unique because it provides a

service termed "factory health services," in which Mary Stopes sets up and staffs a small clinic room within a factory for the nominal fee of 12 Taka per worker per month ($0.21 U.S. per worker per month). Factories that choose to purchase this service pay this amount on behalf of the workers.[9,11,12] The beauty of the service is that it allows a previously neglected group (factory workers) to get quality, convenient health care.

The vast majority of participating factories produce garments.[9,12] Because of their long hours, garment factory workers have very little time to go to hospitals or clinics, especially to government-run facilities, where a patient might spend an entire day waiting to see a doctor.[6] However, the Mary Stopes factory clinic doctor schedules regular checkups for each worker in addition to checkups available on a walk-in basis.[9,11] Workers from participating factories are also eligible to receive free services at any one of the many Mary Stopes clinics available throughout Bangladesh's major cities and can thus get zero-cost referrals to one of these larger clinics.[9] Interviews with factory workers have shown that they very much appreciate the health benefits received from such a setup.[13,14]

Economic Viability of Factory Health Services

Mary Stopes is able to provide these services to factories in a manner that is economically viable to its own organization. By charging 10 Taka/worker/month prior to 2002, it has been able to recover 70 percent of its expenses. In addition, it receives funding from the Department for International Development (DFID), a United Kingdom funding organization, although this funding is scheduled to expire in 2004. As a result, Mary Stopes increased its customer costs to 12 Taka/worker/month in 2002, with the goal of 100 percent cost recovery, and thus eventual self-sustainability of the program.[12]

Appeal of Health Services to Factory Administration

Most factory administrators wholeheartedly support the Mary Stopes health services provided in their factories. They reason that the service cost is very low yet reaps huge economic benefits.[13,14] Workers are more productive because they take fewer days of leave due to debilitating sickness. Workers also feel that their work environment is safe and homey, existing for their benefit.[13,14] As a result, their on-the-job happiness remains greater. These two factors lead to incredible attendance and productivity, as exhibited by the 1 percent absentee rate observed in a garments factory run by director Faruq Hussein, who contracts Mary Stopes factory health services.[13] From a less economic perspective, many factory directors feel a humanitarian obligation to help their poor workers by providing them with health services. Thus, another garment factory director, M. Rahman Razu, began by hiring a doctor and buying medicine with his own money and that of fellow administrators in 1995.

Hundreds of villagers come to see the village doctor.

Dr. Shaukat Jahan, an American volunteer, sees patients in a poor village.

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Later, when he heard of Mary Stopes factory health services, his factory switched to it, like many other factory directors have for its low cost, quality, and reputation.[14]

Factory administrators also enjoy Mary Stopes because it helps protect the factories from quotas that are often imposed due to child labor or poor working conditions. Since Mary Stopes is an international organization, it is highly trusted by international buyers of Bangladeshi-made garments.[12,13] Additionally, by keeping thorough health records of all workers, Mary Stopes can detail the health status of each and every worker.[11] Thus, if need be, such records can dispel myths of poor health and unsanitary factory conditions. Given the prevalence of quotas against Bangladeshi garment factories, especially after September 11, 2001, many factory directors are considering health services such as Mary Stopes with renewed interest.[12]

Appeal of Factory Health Services to Government

In addition to factory workers and administrators, the government approves of Mary Stopes factory health services. The Bangladesh commerce minister, Amir Kusir, looks very favorably on these services,[12] mainly for assistance with quotas. As described previously, quotas are often imposed on factories when working conditions in the factories are suboptimal. The large-scale effect of quotas and the resulting factory closures and production reductions are a general shrinking of the Bangladeshi economy. Like any other government, Bangladesh wants to prevent such negative effects on its already frail economy.[12] This, along with its general efforts to make available better health services that reach a greater portion of the population, makes the government a strong proponent of Mary Stopes factory health services.

Conclusion

Mary Stopes factory health services, a revolutionary system that benefits workers of a leading Bangladesh industry (garments), factory administrators, the government, and the economy, is successfully serving 73,000 factory workers.[9,15,16] Health and efficiency in participating factories is improving dramatically for the miniscule cost of approximately $2.50/worker/year.[9,12] The success of the Mary Stopes program with garment factories has helped it expand to other types of industry, such as fish-processing factories in the city of Khulna.[12] Like garment workers, these workers do not have ready access to health care. Customers, however, do not pressure factories and governments with quotas in any industry quite as much as they do the heavily export-oriented garments industry.[12] Thus, the need for offering health services in other factories seems less pressing to factory owners. This notwithstanding, most factory owners are quickly learning that proper health care can drastically improve worker productivity and lead to greater profits. In addition, other Bangladeshi NGOs such as Momota, which provides health services to some factories in the city of Chittagong, are collaborating with Mary Stopes.[12] Such cooperation among NGOs as well as increased awareness among factory workers, administrators, and government officials will allow factory health services to multiply.

A Mary Stopes slum volunteer describes how to care for a pregnant woman.

Inside the Mary Stopes Dhaka-2 Base Clinic.

The Growing Threat to Research:
Scientific Misconduct

Kelley Rivoire

In the past year, the news that a Bell Labs scientist performing Nobel-quality experiments engaged in scientific fraud sent shock waves through the scientific community. The work of 32-year-old physicist Jan Henrik Schön came under suspicion after he published two papers with an identical figure, including noise patterns. The papers indicated the figures to be measurements of two different phenomena from two different pieces of equipment. One of the papers was published in *Science*, the other in *Nature*. Schön had previously been publishing at the astonishing rate of roughly one paper per eight days, gathering extraordinary results. "He discovered everything in condensed matter physics in the last sixty years" in organic materials, said Lydia Sohn of Princeton University. Later, an independent committee led by Stanford physics professor Malcolm Beasley investigated Schön's work and found Schön guilty of data falsification in sixteen separate instances. [1,2,3] His many co-authors were absolved of all charges, and nearly all of the questioned papers have since been retracted.[3,4,5] With the reproduction of his results having become a "minor industry," others' careers are also at stake.[2] Perhaps most importantly, however, Schön's high-profile case has led to further discussion of the troubling but prevalent issue of scientific fraud and misconduct and the measures necessary to prevent and punish them.

"Nobody arrives at fraud as the first thing they ever do....They got there by doing little things and getting away with it."

Schön Not Alone

Schön's case is hardly an isolated example. In the past five years, instances of questionable scientific research ethics at universities and other research institutions have been numerous. In 2002, physicist Victor Nirov was fired from Lawrence Berkeley National Laboratories (LNBL) in California after his computer analysis of the discovery of elements 116 and 118 was found to be fabricated. Nirov's co-authors, like Schön's, were cleared of any misconduct. The news of his data fabrication also cast a shadow over the discovery of

elements 111 and 112, to whose discoveries he had contributed. The discovery of those elements, however, has not been refuted.[6]

Following are still more examples of American scientists engaging in scientific misconduct. Biochemist Robert Liburdy, also at LNBL, had won more than $3.3 million in federal money for his research on a possible connection between electromagnetic fields and cancer. In 1999, he was found to have fabricated data. He retracted his findings and accepted as punishment the loss of federal funding for three years.[7] In 2001, Harvard medical researcher Evan Dreyer was handed a ten-year ban from receiving National Institutes of Health (NIH) grant funding because of false data reported in papers and grant applications.[8] William Simmons, formerly of the University of Texas Southwestern Medical Center, left his job in 1998 to seek employment elsewhere, only to be called back the following year because the postdoctoral fellow who replaced him could not duplicate his results. When redoing his experiments to prove their validity, he was spotted inserting material into the vials that would alter the results in his favor. His former collaborator, Derry Roopenian, noted that people in his laboratory "wasted a lot of time and money trying to reproduce results that weren't real to begin with," a common complaint of scientists who have based their research on others' fraudulent results.[9]

[Derry Roopenian] noted that people in his laboratory "wasted a lot of time and money trying to reproduce results that weren't real to begin with," a common complaint of scientists who have based their research on others' fraudulent results.

Yet recent scientific misconduct has not been confined to the United States. Overseas, allegations of misconduct are also common. In the field of medicine, Neil Marshall of Britain's General Medical Council (GMC) notes that "we have seen enough complaints to warrant action among the whole profession." In Britain, an article in the *Journal of Bone and Joint Surgery* contained photographs stolen from another author's work. When the author of the article submitted a replacement picture, claiming it as a correction, the new picture was then found to be a combination of two photographs, only one of them from the author's own work.[10] In China, a translator of a 1998 American anthropology textbook decided to publish it in Chinese as well—under his own name.[11] Researchers from two German universities, Professor Friedhelm Hermann of the University of Ulm and Professor Marion Brach of the University of Lübeck, were accused in 1997 of manipulating data in more than thirty biomedical papers. Hermann was later suspended from the university, and Brach lost her position entirely.[12] In Poland, author Marek Wroñski, while writing a book about scientific misconduct in 1997, discovered scientist Andrzej Jendryczko was guilty of more than twenty acts of direct plagiarism. Wroñski claims that an "old guys' network" in Poland protects scientists like Jendryczko from prosecution or defamation. In fact, Wroñski was told that questioning Jendryczko's work "was going to destroy Polish science."[13] Clearly, the problem of scientific misconduct is widespread.

What Exactly Is Scientific Misconduct?

One difficulty in prosecuting scientific fraud often is the lack of a precise definition of the term. The United States has adopted a definition produced by the Office of Science and Technology Policy (OSTP), which states: "Research misconduct is

defined as fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results." This definition, though quite narrow, goes on to pinpoint specific acts that violate good scientific practice, clearing up some confusion in the previous definitions provided by the National Science Foundation (NSF) and Health and Human Services (HHS).[14]

Abroad, some countries also struggle to define misconduct, while others merely focus on how to prevent it. Graeme Catto, vice principal of the University of Aberdeen, claims "there is little value in lengthy discussions about a definition of scientific misconduct as done in the U.S. A better approach seems to be an emphasis on implementing good research practice guidelines."[15] The Wellcome Trust, the largest biomedical charity in the United Kingdom, created its own definition, which recipients of its grants must follow. The definition largely mirrors that of the OSTP but is slightly broader. It is criticized for not providing adequate protection for whistleblowers, but this is partially due to the United Kingdom's strict libel laws. Wellcome hopes that its actions "can become the template for guidelines in other fields of science."[16]

What Measures Have Been Taken to Combat Scientific Fraud?

In 1989, the United States created the Office of Research Integrity (ORI), a branch of the Department of Health and Human Services, to investigate claims of fraud.[17] The ORI receives more than 1,500 complaints a year and often requires ten to twelve months to investigate an allegation, resulting in what Barbara Mishkin, a Washington attorney who specializes in dealing with scientific misconduct, calls a "black-hole effect."[18] More often than not, only lower-level researchers are convicted. The ORI claims a 92 percent success rate, but it has failed several times in high-profile cases. These failures have included false accusations, as in the case of a publication in *Cell* by former MIT professor Thereza Imanishi-Kari, who was supported by Nobel Prize-winner David Baltimore, now president of the California Institute of Technology. Imanishi-Kari was accused of falsifying important data, and her case required a full decade to resolve before she was cleared of wrongdoing.[18,19] Nearly all universities and research institutions also conduct internal reviews whenever a scientist comes under fire. In addition, in the United States, the NIH now requires medical schools to provide training in research ethics, and some universities have expanded such classes to all graduate students.[20,21]

In Europe and Asia, methods of dealing with scientific misconduct have also been discussed at length. The Chinese Association of Science and Technology has issued a number of rules for science journalists, requesting journals not to publish multiple submissions or papers of poor quality and forbidding the publication of papers dealing with confidential information.[22] In the early 1990s, Denmark created the Committee of Scientific Dishonesty (CSD), which has considerable power over scientists because the Danish government funds more than 90 percent of the country's research.[15] Rather than develop a national body to investigate misconduct, other countries have preferred to simply tie funding by national organizations to compliance with misconduct procedures. In Australia, the National Health and Medical Research Council has

"Many researchers think that a high IQ goes hand in hand with high moral values." ...[T]his correlation "is, of course, absolute nonsense."

guidelines to which grant-receiving research institutions must adhere.[23] The German Deutsche Forschungsgemeinschaft, the nation's largest source of grants for basic research, has proposed ways to promote research ethics and deny funding to those who do not comply. Among their recommendations are requiring the storage of original research data for ten years, offering protection to whistleblowers, and eliminating the practice of honorary co-authorship. In addition, prominent research institutions all over the world, such as the Max Planck Society in Germany, have been preparing their own procedures for dealing with misconduct. President Hubert Markl hopes "universities or other institutions will look closely at our new rules and perhaps use them as an example."[12] The only question that remains is how successful these agencies can be at finding and punishing misconduct, as well as preventing it.

The high-pressure environment that today's young researchers enter can often tempt them to practice poor ethics. More than 1 percent of scientists report direct knowledge of an instance of misconduct.

Why Would Anyone Commit Scientific Fraud?

In the words of Stephen Lock, a past editor of the *British Medical Journal*, "Many researchers think that a high IQ goes hand in hand with high moral values." However, he continues, this correlation "is, of course, absolute nonsense."[15] The high-pressure environment that today's young researchers enter can often tempt them to practice poor ethics. More than 1 percent of scientists report direct knowledge of an instance of misconduct.[21] The "publish-or-perish" mindset of research institutions that often base promotions on the volume of research published can lead a young researcher astray, particularly in the extremely competitive and lucrative biomedical industry.[17,24] Because of the huge volumes of research produced in a year, many also believe their questionable activities will escape notice. Computer programs can make falsifying images such as X-rays easy and indiscernible from genuine ones.[10] A person who successfully passes off small data manipulations may attempt misconduct on a larger scale the next time. "Nobody arrives at fraud as the first thing they ever do....They got there by doing little things and getting away with it. Calling them in might stop people from going off the deep end," said Paul Friedman of the University of California at San Diego.[17] In addition, the system of tenure in universities can lead to a "cloak of academic freedom," where a professor's research may not undergo the necessary internal scrutiny before publication, because professors "invariably resist intrusion from their fellow departmental colleagues."[25] The environment of the research setting seems to push researchers to produce results at any cost.

Who Is to Blame?

The issue of responsibility for fraudulent papers has been much disputed recently. Those often mentioned as culpable include co-authors, supervisors, and peer reviewers. With multiple authors from multiple countries working in cross-disciplinary fields, it is hard to hold each person accountable for every piece of data or analysis reported in a paper. In the Schön case, when co-authors were questioned as to the particulars of data measurements, they professed to know little. Yet it can be argued that, since each receives part of the credit for a successful paper, each should bear responsibility for the contents. As Donald Kennedy of *Science* remarked, "If

the benefits of authorship are enjoyed jointly and severally by all the authors, shouldn't the liability be shared in the same way?"[20]

Supervisors and management are also often blamed for failing to catch errors before an article goes to press. While some accountability for the results produced is expected of a supervisor, it is unreasonable to expect 100 percent overseeing of a project. Where should the line be drawn? Finally, no one has ever claimed that the peer review process is perfect. Unless an article is in their particular field of research, reviewers are unlikely to question the validity of a paper. Furthermore, while peer review is an anonymous process, a reviewer would be identified and subject to legal action if he or she were to accuse an author of misconduct.[25] As Donald Kennedy of *Science* wrote, "A clever laboratory cook can invent data that are immune to vigilant reviewers and to any diagnostic test, save repetition, the only proven scientific remedy."[20] However, increasingly complex research means that duplicating another's experiment to ascertain its validity can be phenomenally costly and time-consuming, if not downright impossible.

What To Do Next?

The ORI has several plans in the making, but all have been met with stiff resistance. Its funded proposal to investigate those found guilty of scientific misconduct to learn about their mentalities and what caused them to behave in such a way was halted by the Office of Management and Budget (OMB) after the completion of the first stage.[26] The ORI also proposed a Gallup survey to send to 3,000 researchers to further define standards of ethical research conduct. The survey required the scientists to answer questions dealing with improper citation of articles and poor research supervision, but this approach has been attacked as too broad. Critics also feel that the ORI may be exceeding its limits to areas not included in the OSTP definition of scientific misconduct.[26,27,28] The president of the Federation of American Societies for Experiment Biology (FASEB), Stephen Teitelbaum, calls the survey "a terrible instrument" and its questions "outrageous."[26]

"If the benefits of authorship are enjoyed jointly and severally by all the authors, shouldn't the liability be shared in the same way?"

In Britain, the General Medical Council plans to specify both the standards for research practice and the punishments for those who violate them.[10] Individual researchers have also developed their own ways to deal with issues of fraud, such as checking the work of their associates more carefully.[21] Some supervisors even feel unable to reveal the identity of reagents to researchers working in their laboratories for fear of data falsification.[15] The Institute of Medicine has also suggested continuation and expansion of research ethics training, though Arthur Rubenstein of the University of Pennsylvania, the panel's chair, anticipates "quite a lot of flak" on the controversial idea. Rubenstein even goes so far as to recommend ethical conduct as a part of promotion considerations and research ethics programs as a requirement for university accreditation.[27] Even with ethics courses, as Hubert Markl of the Max Planck Institute remarked, "One shouldn't expect too much from formal

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[ethics] courses; there are tons of lawyers who finish their formal legal training and still go work for the mob." Instead of merely endorsing ethical practices, "You have to teach it by example; the young scientists have to see it day in, day out," stated Lord Kilpatrick of Kincraig, a past president of the British General Medical Council.[15]

As to the best way to prevent and punish scientific misconduct, there seems to be no clear answer. Awareness of the problem alone is a huge step forward, but it is certainly not enough. Perhaps the best suggestion comes from Paul Grant, of *Nature*—for colleagues to educate each other about their work, to the level that any co-author can defend another's work. Not only does this reduce likelihood of fraud, it also allows scientists to interact with one another to expand "technical vitality."[30] Without any additional supervision for each and every researcher, the problem of scientific misconduct will not go away. In the words of Cai Decheng, former vice president of the Chinese *Science and Technology Guide*, "There must be no compromise over dishonesty and no cover-up. Taking pity will harm the cause of science."[16]

Cloning:
Where We Have Been and Where We Will Go from Here

W. Victoria Lee

In *The Devil's Dictionary,* American author and satirist Ambrose Bierce defines admiration as "our polite recognition of another's resemblance to ourselves." Indeed, we all love ourselves. There are a few others whom we can love to the same extent: our parents, our significant others, and our children. But can we also love our clones, exact genetic replicas of ourselves? That is, if we were ever to have clones. This seems like a very distant possibility. However, we may not be too far away from a world where we coexist with our clones.

At the dawn of 2003, Brigitte Boisselier, a French chemist, claimed the birth of the first human clone nickednamed Baby Eve. Boisselier was also busy running the first human cloning company, Clonaid, and serving as the bishop of the Raelian religion, a new religious sect that believes aliens created life on earth, that sexual freedom is important, and that life can be eternalized through cloning technologies.[1] She managed to shake the scientific community, add fuel to the already burning cloning debate in the U.S., and stir a bigger wave of controversy among religious groups with her evidence-deficient claim before the festive New Year.

Scientific technology has advanced rapidly over the past six years since the birth of Dolly, the first cloned mammal, in 1996. The question used to be: "Is it possible to clone?" The question today is: "To clone or not to clone?" In just a few years, an array of different animals has been cloned as the technology has continuously improved. We can now look back at what we have accomplished and ask ourselves, "Where we will go from here?"

Although not an easy thing to do, cloning has proved to be contagious. Now there are hundreds of cloned animals, including sheep, goats, mice, pigs, cows, cats, and, most recently, rabbits.

The Journey So Far

Dolly: The Cloned Sheep

The hype about cloning began with the birth of Dolly, the first cloned mammal born on July 5, 1996, at the Roslin Institute in Edinburgh, Scotland.[2] The project was headed by Dr. Ian

Wilmut and his team of embryologists. Their success not only opened a new frontier in biological possibilities but also initiated the first wave of debate on the ethics of cloning.

The fact that cloned mice are attainable indicates that DNA can be reprogrammed in a short amount of time, which suggests that a cloned human can be created.

Dolly was cloned from an udder cell of a 6-year-old Finn Dorset ewe.[3] Out of 250 attempts and twenty-nine cloned embryos created by the team, only Dolly developed successfully, a first sign indicating that cloning is not an easy business.[4] Nevertheless, Dolly was able to mate normally and give birth to healthy lambs, proving that clones are reproductively capable. A clone's life, however, is not easy. Besides being under constant attention, Dolly developed arthritis, obesity, and lung complications that rendered her a rather uncomfortable life. As a result, Dolly was euthanized in mid-February this year at the age of 6, about half of an average sheep's life expectancy.[3]

Before a postmortem autopsy is performed, it is hard to tell if Dolly's declined health was a result of her being a clone. After all, Dolly was not an ordinary sheep. As a celebrity animal, she spent most of her time indoors; a factor that scientists now suspect might have contributed to her lung failure. Her other health-related disorders such as arthritis and obesity have also been considered side effects of her constantly begging for treats and standing on her hind legs.[5]

Some researchers, however, have found some scientific evidence suggesting that her arthritis and her lung problem, a common lung disease seen in indoor elder sheep, were due to her being a clone.[5] A study conducted when Dolly was 3 revealed that Dolly was in fact older than thought. At the tips of the chromosomes there is a region called the telomeres, which usually becomes shorter as cells continue to divide and develop. Scientists found Dolly's telomeres much shorter than other sheep of her age. Although only 3 years old when the test was conducted, her telomeres were similar to those in a 6-year-old sheep, an indication that Dolly could have had a shorter life expectancy.[6] The decision to put her to death this past February because of her failing health might have validated this omen.

Cloned Mice

Almost two years after Dolly's birth, scientists in the laboratory of Ryuzo Yanagimachi at the University of Hawaii successfully created cloned mice. Even more astounding is the fact that the cloned mice were produced using a different technique than that which produced Dolly, indicating that more than one way exists to clone animals. A drawback to the technique is that it requires types of cells found only in female bodies. As a result, the technique only works on females. Like Dolly, the first cloned mouse that successfully reached adulthood was also given a name, Cumulina. Born on October 3, 1998, it was among more than fifty cloned mice produced after hundreds of attempts. Similar to Wilmut's experiment, the success rate for cloning was much lower than one might have expected; only about 1 to 2 percent of the clones were born. However, the team was also able to produce second-generation cloned mice, clones of clones.[7]

A more important aspect of the team's experiment is that the animal cloned was a mouse. Experts previously thought that Dolly was a success because she was a sheep. Many farm animals have cellular DNA intervention fairly late in embryonic development. The delay buys time for the implanted genetic material "to be reprogrammed by the egg."[7] However, DNA starts doing its work very early in mice and human embryonic development. The early intervention would require functional DNA that has already been "properly programmed."[7] The fact that cloned mice are attainable indicates that DNA can be reprogrammed in a short amount of time, which suggests that a cloned human can be created.

Cloned Human Embryo

A Worcester, Mass.–based company, Advanced Cell Technology (ACT), announced in late November 2001 that it had successfully cloned human embryos. By using the

standard cloning technique (as explained in the next section), the company was able to create a six-cell human embryo.[8] The news invited excitement, outrage, and skepticism. Many scientists thought the announcement was rather hasty and premature, because a mere six-cell embryo is a far cry from a clone. According to experts, an ovum is capable of developing through the eight-cell stage "without any signals from the DNA in the nucleus."[9] Therefore, it is possible that what ACT observed is not the genuine success of cloned embryos.

ACT is not new to the cloning business. The company was known to have created an embryo by inserting human DNA into a cow's ovum. The high degree of reaction to such a quasi-minotaur creation is to be expected. But the company only allowed the embryo to grow through five cell divisions, as the researchers' goal was not to create an actual human clone but to experiment with possibilities in stem cell research.[10]

The company also experimented with parthenogenesis, a process in which a human ovum is allowed to "divide into early embryos without being fertilized by a sperm or being enucleated and injected with a donor cell." The goal of this was to obtain stem cells to grow transplantable organs that are less likely to be rejected by patients. Although previous experiments done by other experts with eggs from other animals have proven successful, ACT was not able to produce successful embryos that contain stem cells.[11]

Cloned Pet

Although not an easy thing to do, cloning has proved to be contagious. Now there are hundreds of cloned animals, including sheep, goats, mice, pigs, cows, cats, and, most recently, rabbits.[5] The famous first cloned pet, a cat named Cc, was born at the end of 2001 in a laboratory in Texas. Cc was the only one to survive out of eighty-seven cloned embryos. Not unlike other cloning success stories, Cc's birth also generated immense excitement and controversy. Experts are now looking into cloning technology to save endangered species; a type of endangered wild cattle has already been cloned successfully. Businessmen are also hoping that one day cloning technology can be used commercially to allow pet owners to re-create their deceased beloved companions.[12] Although many types of animals have already been cloned so, animals such as dogs, certain monkeys, and human beings have yet to be replicated.[5]

Cloned Human?

Baby Eve is allegedly a clone of her 31-year-old American mother made from her skin cell and an ovum. She was the first of the five cloned babies claimed by Clonaid to be born.[1] All babies were due to be born by early February 2003, including the baby of a Dutch lesbian couple. Although Boisselier maintains that the birth of the cloned babies is a new scientific breakthrough, most experts are highly skeptical of her claim. Not only does Boisselier have no evidence to prove the baby's existence or the fact that she's a clone, but she has also backed off from having the baby tested for authenticity because of her concerns for the safety of the baby. The fact that Clonaid has not cloned anything before and that their success rate of the claimed human clone—five in ten—gave the public legitimate reason to doubt. But as what Robert P. Lanza, a cloning expert with ACT, points out, "It may be rather easier than any of us thought to clone a human."[13] Maybe, just maybe, we are all looking in the wrong direction.

With the low success rate of producing a viable cloned embryo, it is legitimate to question how we will be able to obtain hundreds of eggs just to produce one or two cloned embryos.

The Process

Cloning, another term for making an exact genetic replica, is not unnatural in the biological field. Many organisms rely on cloning to continue their existence. Cloning, however, is an artificial novelty in animals, especially mammals. Decades ago, it was fictional or even fanatical to talk about cloning, making a genetic copy of an animal, let alone a human. Are we magicians, are we miracle workers, or are we Dr.

Frankenstein reborn? How does cloning really work?

A generic cloning technology, somatic-cell nuclear transfer, provides the basic framework for other different cloning techniques. Somatic cells are the so-called body cells. In the process of somatic-cell transfer, a somatic cell's nucleus, the part of a cell that contains the DNA, is extracted and inserted into an enucleated ovum. The combined entity will develop into an embryo that will then carry the DNA of the somatic cell and therefore the same gene as the donor of the somatic cell in every one of the new embryo's cells.[6] The exact process, of course, is not so simple. The ovum, or egg as it is commonly known, first needs to be placed in a culture dish until it matures, while being soaked in a chemical bath, and then its genetic material is removed. Everything is also done on a microscopic level, which makes the process extremely painstaking. In order to enucleate the ovum, the researchers have to first remove a small portion of a protective layer, called the "zona pellucida," on the outside of the ovum. To do this, a microscopic needle is used to "drill" out the small piece of the layer, called a plug, while a pipette is holding onto the ovum. After the plug is removed, the polar body and the genetic material in the ovum are sucked out with a needle. The nucleus or sometimes the entire somatic cell is then inserted into the ovum through the "hole" created. The resulting entity is then placed in a chemical bath that will stimulate it to divide and develop.[14]

Dr. Jeffrey Kahn... points out that the majority of indignation against therapeutic cloning is because most people think "it is unethical to create human life that will be destroyed."

Dolly was produced using the somatic cell from a ewe. The specific technique used by Wilmut's team was the fusion of the entire udder cell and the enucleated ovum by electrical jolt. The cloned mice, on the other hand, were produced using cumulus cells, which nourish and cling to the ova on the outside. In addition, a cumulus cell was inserted into the ovum by injection rather than electrical fusion. Replacing just any somatic cell with a cumulus cell is also significant in that cumulus cells are found on human ova as well.[7]

From the findings of cloned mice, ACT was able to use two techniques to make the human cloned embryos. The company injected nuclei of skin cells into some ova and cumulus cells into others. Like other cloning experiments, it took many attempts before they were able to produce a successful cloned embryo from the ovum that was injected with cumulus cells.[11]

The Moral Dilemma

We have longed to do it, and now we have done it. But what remains is the moral question of the line between therapeutic cloning and reproductive cloning. Therapeutic cloning explains why we wanted to clone in the first place.

The original goal of Dr. Wilmut and his team of scientists was purely therapeutic. They made Dolly in the hope that the experiment would lead to the mass production of genetically engineered farm animals whose organs could be used for transplantation or whose milk would have medicinal purposes for sick patients.[2] Yanagimachi's team and ACT's researchers have also had similar goals in mind when they conducted their studies.

Therapeutic Cloning

"Therapeutic cloning" is a term coined to describe the process of harvesting stem cells from a cloned embryo, which "is made using the DNA of a patient who could benefit from a stem cell transplant."[15] The harvested stem cells can then be developed into the specific tissue, organs, or even nerves that the patient needs. A wide range of disorders can be cured with the compatible body parts grown from stem cells, including diabetes, Parkinson's disease, Alzheimer's disease, stroke, and epilepsy. It is even possible to create blood cells and bone marrow from stem cells with proper cell differentiation.[11]

The ultimate goal is laudable, but it is the process of harvesting the stem cells that has

generated an immense wave of controversy—a hot topic excessively discussed in the abortion issue, namely the debate of whether an embryo is a human being or just a bunch of cells.

Dr. Jeffrey Kahn, the director of the Center for Bioethics at the University of Minnesota, points out that the majority of indignation against therapeutic cloning is because most people think "it is unethical to create human life that will be destroyed."[16] Indeed, the people who are enraged by such scientific attempts range from religious to political leaders. When ACT first announced its human embryo in 2001, President Bush had already expressed his strong disapproval. He maintained that "we should not as a society grow life to destroy it."[17] Although Archbishop Tarcisio Bertone praised the therapeutic aims of cloning, he insisted that "if the process involves production and destruction of human beings to treat other human beings, then the end doesn't justify the means."[8]

Other scientists and religious leaders, such as Cynthia Cohen, a researcher at the Kennedy Institute of Ethics at Georgetown University, have come to the conclusion that any embryo "younger than 14 days cannot be considered human because cells have not formed a single individualized entity."[18] For some, then, research dealing with cloned embryos no older than 14 days old is therefore acceptable and ethical.

As it turns out, some scientists also hold a very skeptical view on how much embryonic cloning will modernize stem cell therapy. There are millions of people suffering from diseases that require various transplantations, but as Harry Griffin, assistant director at the Roslin Institute, points out, "There are simply not enough human eggs available" to produce cloned embryos from which to harvest stem cells.[9] With the low success rate of producing a viable cloned embryo, it is legitimate to question how we will be able to obtain hundreds of eggs just to produce one or two cloned embryos.[6]

Reproductive Cloning

Another aspect of cloning research that is belittled by politicians and scientists alike is reproductive cloning. The process is essentially the same as that of therapeutic cloning in terms of creating the embryo. In reproductive cloning, however, the embryo is planted into a woman's womb and developed into a fetus. But with in vitro fertilization and other infertility treatment readily available, why would one turn to cloning for babies? It turns out that people who don't have ova, sperm, or either can use cloning to produce a child that is genetically related (more accurately, identical) to them by taking one parent's somatic cell nucleus and inserting it into a donor egg that does not contribute to the genetic make-up of the embryo.[6]

Although these applications of cloning make the technology seem like a panacea to infertility and genetic disease-related problems, from a bioethical point of view, reproductive cloning is really the fountainhead of all moral predicaments.

Other candidates who might benefit from reproductive cloning are lesbian couples who don't want their children to carry the genes of unrelated sperm donors. Couples who are genetic disease carriers might also want to use cloning to prevent having children with a full-blown version of their diseases that might result from the genetic mixing during sexual reproduction. Furthermore, these parents can have gene therapy applied to their diseased somatic cell before its nucleus is inserted into the egg to form an embryo, thereby forever obliterating the diseased gene from the family tree.[6]

Although these applications of cloning make the technology seem like a panacea to infertility and genetic disease-related problems, from a bioethical point of view, reproductive cloning is really the fountainhead of all moral predicaments.

One potential problem has to do with the clone's identity. Because the clone has the same DNA as its somatic cell nucleus donor, the two are essentially identical twins. As a result, one "could give birth to [one's] own twin, or the twin of [one's] mother or

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Because the clone has the same DNA as its somatic cell nucleus donor, the two are essentially identical twins. As a result, one "could give birth to [one's] own twin, or the twin of [one's] mother or father."

father."[16] Although people are made up of more than genes and environmental factors play a significant role in their personality and appearance, it is expected that clones, however different they might be from their adult "originals," would encounter difficulties in psychological development. The cloned child might be expected to live a life similar to the "original" and might be looked down on if he or she fails to do so. The cloned children might also have low self-esteem because they are copies of someone else. They might lack motivation to control their own life because they already know certain traits from their adult "originals" that they are destined to bear, such as shortness or baldness.

Furthermore, will we move from reproductive cloning to selective cloning? Will we choose to clone only the beautiful, the healthy, the strong, and the smart adults among us? As Robert Wachbroit points out in "Genetic Encores: The Ethics of Human Cloning," we know some newborns will lead difficult lives such as the children who are born into poverty, "but we don't thereby conclude that such children shouldn't be born."[19] These children might not have material luxury, but they can still enjoy loving families. How do we decide who should be cloned? And by performing artificial selection, are we meddling with Mother Nature? What would become of human evolution?[19]

These questions may appear far-fetched and even unrealistic right now, but they are legitimate concerns once we start cloning humans as frequently as we use in vitro fertilization today.

But before we worry ourselves sick with these ethical questions, we should ask if human cloning is safe. As it turns out, human cloning might be a dream too good to be true. Professor Rudolf Jaenisch at the Whitehead Institute for Biomedical Research at MIT and his team reported in last September's issue of *Proceedings of the National Academy of Sciences* that "the cloning process jeopardizes the integrity of an animal's entire genetic make-up."[20] Having studied around 10,000 genes, researchers found that in cloned mice's placentas as much as 4 percent of the genes is abnormal.[20] Two other studies also showed that the path to human cloning might not be as bright as we thought. Researchers from the University of Connecticut in Storrs have been studying the X chromosomes in unsuccessfully cloned cows and found that some genes on the chromosomes are not expressed. Researchers at the University of Pennsylvania have also discovered that a fate-determining gene called Oct4 goes awry in more than 90 percent of the cloned mouse embryos.[21]

These findings not only put a stop sign in the middle of the human cloning rush but also raise doubts about the safety of stem cells harvested from cloned embryos. Researchers like Jaenish, however, have confidence in the safety of therapeutic cloning because tissues or organs grown from such stem cells "would not contribute to the development of [the] whole organism."[21]

What Do the Politicians Say?

They say "no" to cloning, period. Reproductive cloning has never received much support, but people have also been looking askance at therapeutic cloning, trying to nudge it out of the picture inch by inch.

Long before Dolly was born, the talk of human cloning had already begun in the scientific community. By the end of 1994, the National Institutes of Health (NIH) decided to prohibit human cloning research but expressed desire to permit federal funding for some human embryo research that might lead to the discovery of treatments for terminal diseases. President Clinton, however, thought otherwise and prohibited NIH to fund human embryo research.[2] When Dolly's birth was announced, President Clinton took it one step further and established a moratorium on all human cloning experiments with federal funds. At the same time, the National Bioethics Advisory Commission wanted a ban on private research as well.[6] When President Bush asked Congress to ban cloning in 2002, the House of Representatives agreed with him and introduced the bill again as soon as Boisselier announced Baby Eve's birth. As of mid-February, the cloning ban has been endorsed by the House Judiciary Committee, pending floor debate and vote.[22]

While lawmakers unanimously agree to place restrictions on cloning research, the Republicans seem to be more uncompromising than their Democrat colleagues. They are seeking to pass a bill in the Senate that not only puts a stop to cloning attempts but also to the creation of embryos for any purpose. Democrats, on the other hand, hope to exempt therapeutic cloning research in their own bill.[18]

Cloning is such a familiar yet strange word. We have come a long way, yet there is still so much unknown. Surely scientists will one day figure it all out. The question is: How will we deal with the moral issues of the findings? And in the meantime, will legal issues stand in the way? As Dr. Kahn points out, "It is not cloning techniques that are unethical, but some of their potential applications."[15] Ultimately, what the future of cloning will be is a question that only time can answer.

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Stem Cell Research:

The Debate that Has Divided America

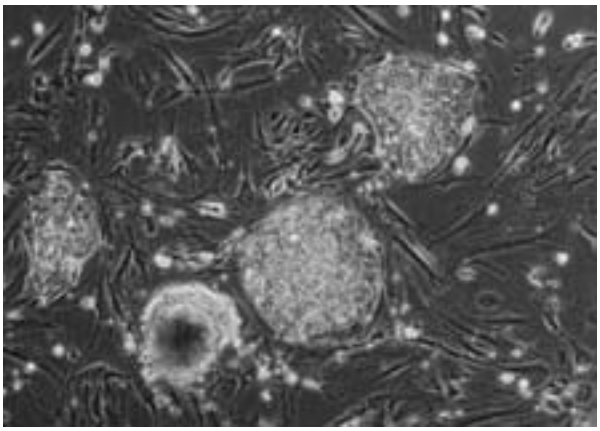
Farhan Merali

A scenario:

You have severe congestive heart failure. You need a heart transplant, but your doctor tells you that due to the scarcity of replacement hearts, you might have to wait a year or more. After the transplant, your body's immune system might still reject the new heart. Even if the transplant is a success, you will have to endure the effects of immunosuppressive drugs for the rest of your life.

Another scenario, this one for the future:

*You have severe congestive heart failure. Your doctor admits you to the hospital. During an operation later that day, she injects some heart cells into your heart, and after an integration period of a few days, your heart is healed substantially. You go home and lead a healthy life.**



Microscopic 10x view of a colony of undifferentiated human embryonic stems cells being studied in developmental biologist James Thomson's research lab. The embryonic stem cell colonies are the rounded, dense masses of cells. The flat, elongated cells in between the embryonic stem cell colonies are fibroblasts that are used as a "feeder layer" on which the embryonic stem cells are grown. (Source: University of Wisconsin-Madison.)

Doesn't the second scenario sound better? What if you knew that the heart cells were generated from human stem cells, taken from human embryos? Depending on your belief about when life begins, this might bother you a great deal or it might not bother you at all.

Stem cells are the ordinary undeveloped cells of very early–stage (no more than 64-celled) embryos.[1] To put this in perspective, a newborn baby is composed of billions of cells. Many of these embryos have been grown in a laboratory from fertilized eggs; they were produced for in-vitro fertilization but were later discarded or donated specifically for research purposes. Embryonic stem cells are pluripotent—unlike more mature cells, they hold the possibility of developing into any organ of the body. Scientists experimenting with mice have introduced pluripotent mouse embryo stem cells into diseased organs. These stem cells then begin to take on certain characteristics and functions of the organ cells. The stem cells don't actually develop into organs, but they do begin to resemble the organ cells; stem cells introduced into a diseased kidney, for example, mime ordinary kidney cells. The other kidney cells integrate the new cells until the organ is effectively regenerated.[2]

* Scenarios have been adapted from Horvath, 2003.

Culture trays containing human embryonic stems cells being viewed under a microscope and studied by developmental biologist James Thomson's research lab.
Photo by: Jeff Miller

The second of the given scenarios is what researchers hope will become an everyday reality early in the 21st century, not only to treat diseased hearts but also to treat damaged livers, kidneys, and lungs as well as neurological diseases. By the very nature of the research, however, scientists have found themselves entangled in a moral and ethical debate that has divided America: Should stem cells from embryos that could potentially develop into living beings be used to treat a wide array of diseases? The answer is contingent two main parts: When does a fertilized human egg become a living person? and Do the benefits of the therapy warrant the controversial use of stem cells?

Stem cells currently being used for research purposes and experimental studies are usually derived from aborted fetuses or embryos that have grown in a laboratory from fertilized eggs. The eggs used are normally produced for in-vitro fertilization, a process that helps many women to conceive children. When doctors match sperm and egg to create embryos outside the womb, they usually produce more embryos than are planted in the mother. These embryos are later discarded or donated specifically for research purposes. Some Pro-Life activists argue that an egg becomes a living person the instant it is fertilized; some scientists argue the opposite, using the following logic: The fertilized eggs used for research purposes sit in vats of liquid nitrogen at subzero temperatures until they are otherwise thrown out. The eggs have the *potential* to become living beings, but they will never be implanted inside a woman's uterus to undergo the process of developing into a living, breathing human being. How, then, can these be called "human beings"? The same holds true for aborted fetuses: The decision to prevent the fertilized egg from becoming a living being has already been made.

Others argue that the question boils down to one of intent: What was intended to become of the eggs? If the egg in question were in fact implanted inside a woman, then it would have the opportunity to undergo the process of development into a human being. However, if the egg in question is one of the 30,000 leftover eggs*[1] which couples don't need after they've had their child and which are set aside to be discarded every year, then the intended path is *not* one of life. For such an egg, the decision regarding the potential to become a living being has already been made. Why, then, scientists argue, should such an egg be prevented from aiding researchers to find potentially life-saving treatments using stem cell technology? The same holds true for eggs donated for research, as well as aborted fetuses from which stem cells can be harvested.

The end results of utilizing embryonic stem cell research are numerous and highly promising: They could lead to life-saving therapies for Alzheimer's disease and diabetes as well as to ways to prevent birth defects and rebuild damaged organs. For example, in the 30 July 1999 edition of *Science* magazine, scientists reported how they were able to manipulate stem cells into neural cells and inject them into fetal or newborn rats who have a disease in which the myelin coating around nerve fibers is missing.[3] The cells had been developed into key cells of the nervous system that were able to promote the growth of myelin covering to help nerves function normally. These tests show promise for a viable treat-

* Figure represents numbers in the United States alone.

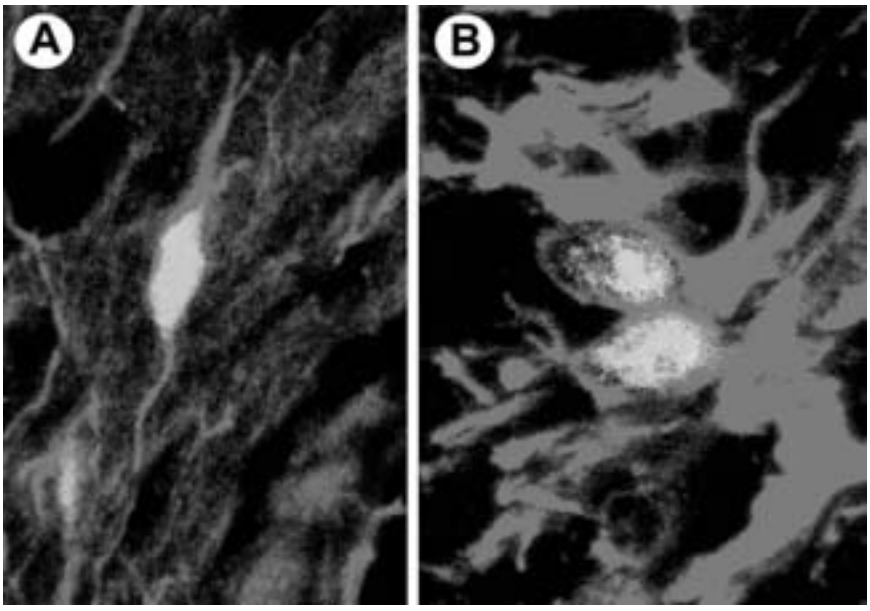
ment for multiple sclerosis (MS), a disease in humans that parallels this demyelination in rats, for which there is currently no cure.

Geron, a small biotech firm in California, is working on a project that could profoundly change medical treatment by offering patients customized parts to repair damaged organs. The Geron Corporation has developed a technology called "telomerase expression," which it says allows cells to keep replicating. With this technology, in conjunction with stem cell technology, Geron researchers would be able to grow new human tissue capable of repairing heart muscle, bones, nerves, skin, and eyes.[4]

Though mainstream media sometimes gives the impression that the public will have to wait years before revolutionary treatments involving stem cell technology for human diseases become a reality, this is not the case. This past July marked the twenty-first month of "complete clinical remission of [lupus]" in an 18-year-old woman who was so near death from the disease that she needed life support because her lungs and kidneys were failing. Now—about two years later—she is in good health and showing no signs of the disease. The young woman became one of a handful of lupus patients to undergo a stem cell treatment that may cure the disease.[5]

What if the government had decided to halt stem cell research before this revolutionary treatment for lupus had come to fruition? Can one justify depriving an individual who has a life-threatening illness of a potentially health-restoring treatment by utilizing an embryo that was set aside to be discarded anyway? Again the question is, When does a fertilized egg become a human being? Even if this line is unclear, can one justify preventing scientists from conducting research that could lead to and has already shown promise in treatments that can save thousands, if not millions of lives with cells derived from these same eggs? No one knows exactly how many lives could be saved, but if one examines the number of people afflicted with diseases for which stem cell therapy could potentially be a cure, the numbers are staggering: 1.4 million people with lupus,[5] about 1 million people* with Parkinson's disease,[6] and approximately 220,000 people with spinal injuries, with about 10,000 new injuries per year.[7] These figures represent the number of people afflicted in the United States alone, and do not even include the countless other people with disorders that could be cured or improved with a regenerated body part or organ.

One of the most promising frontiers of science has rocked America with one of the greatest moral dilemmas: Should these "master cells" that are present only in early-stage human embryos be used for eventual life-saving therapies for Alzheimer's disease, Parkinson's disease, multiple sclerosis, and other neurological and physiological disorders, as well as to prevent birth defects and rebuild damaged organs? Some people may have difficulty with weighing life-saving medical benefits against moral costs, but some argue that there does not need to be *any* moral cost. Advocates of this argument posit that the much-debated "line" between when a fertilized egg becomes a living being does not need to be delimited. Again, they say the question boils down to one of intent. The implications of stem cell technology are very real and have already saved several



After transplantation into the brains of young mice, the neural precursor cells give rise to functioning neurons (A) and astrocytes (B), a star-shaped cell of the brain and spinal cord. Photo courtesy of Su-Chun Zhang

* The number is approximate because many people, perhaps half of those affected, are thought to be undiagnosed.

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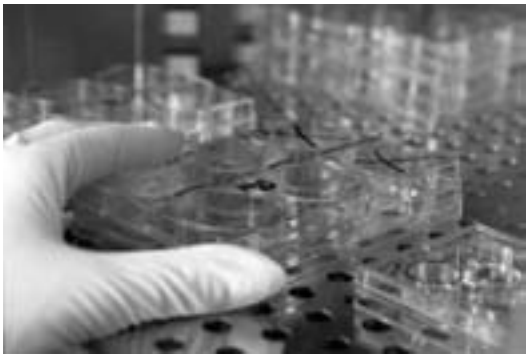

Culture trays containing human embryonic stems cells being stored in heat-controlled storage and studied by developmental biologist James Thomson's research lab. Photo by: Jeff Miller

human lives as well as shown promise in animal models. Halting stem cell research could potentially destroy the light of hope for the millions of people in the United States whose lives could one day be improved or even saved through the use of stem cell technology. Many agree that it is wrong to create human embryos in test tubes solely to experiment on them, but if the remaining eggs from an in-vitro fertilization are to be discarded regardless, they could be used instead to cure a child with diabetes, a 65-year-old with Alzheimer's disease, or a young father with a paralyzing injury. An embryo has the potential for life at one point, but if this route is no longer the intended one, stem cell technology gives an embryo the potential to *save a life*. Perhaps, then, we as a society must decide the point at which the *potential* for life can be outweighed by our need to survive. Then, if we found ourselves facing the opening scenario, would we change our minds?

The Spectroscopic Determination of Aqueous Sulfite Using Ellman's Reagent

Cameron Sadegh and Ronald P. Schreck
Academy for the Advancement of Science and Technology, Hackensack, NJ

Abstract

At room temperature and in N_2-purged pH 8 aqueous phosphate buffer, the sulfite ion (SO_3^{-2}) cleaves the disulfide bond in Ellman's reagent, 5-5¢-Dithiobis-(2-nitrobenzoic acid), and displaces one of the chromophoric anions, producing a yellow hue. Absorption spectroscopy of the resulting chromophore, 5-mercapto-2-nitrobenzoate, permits a quantitative assay of the initial sulfite concentration using a linear relationship in accordance with the Beer-Lambert law. This colorimetric method was validated with several test reactions and was determined to be accurate to within a 2% average relative error. Its sensitivity has been demonstrated down to 0.8ppm sulfite (10^{-5}M). One drawback of Ellman's reagent is that it will react with other compounds containing thiol groups; yet, in some interesting cases, such as in the water boiler industry, other such compounds are absent and this technique would provide a reliable, inexpensive, and field-usable method for determining sulfite concentration.

Introduction

Sulfur-oxy anions play an important role in our nutrition and environment. One such anion, the trigonal pyramidal sulfite (SO_3^{-2}), has several fascinating and uncommon uses. Sulfites are currently used to control microbial growth, bleach certain food starches, and prevent spoilage of certain perishable foods, beverages, and pharmaceuticals. Several examples of where sulfites are used include shrimp, dried apricots, dried raisins, lettuce and other vegetables, and potato chips. Furthermore, their antioxidant and antimicrobial properties play an important role in wine-making. The sulfites either inhibit or kill bacteria and wild

yeast, thus encouraging rapid and clean fermentation of wine grapes. The paper and pulp industries use sulfites as additives to improve strength, increase brightness, and lower pulping energy. The photographic industry uses sulfite for the testing of fixing baths, stop baths, and developers. Sulfite is used in the water boiler industry as an oxygen scavenger, which binds well with excess oxygen to form sulfate. Yet sulfite concentrations in boiler and process waters must be monitored routinely to avoid overtreatment, which can lower the pH and cause rust. Waste treatment plants remove residual chlorine in wastewater by injection of either sulfur dioxide gas or a solution of sodium sulfite or sodium bisulfite (strong reducing agents). In practice, most plants subject to dechlorination requirements run relatively high-sulfite residuals to ensure complete chlorine removal at all times. However, excess sulfite in wastewater effluents may be harmful to marine life. Because sulfites are widely used in industries, a method for monitoring its levels is a necessity for environmental safety.

Sulfite may also be harmful to some humans with allergies. A 1985 study by the Federation of American Societies for Experimental Biology found that in some patients with asthma, ingesting sulfites might lead to an acute and sometimes life-threatening attack of asthma. This study prompted the U.S. Food and Drug Administration (FDA) to issue regulations prohibiting the use of sulfites on fresh fruits and vegetables that are usually eaten raw. In addition, the FDA now requires other foods, such as wine, to indicate the presence of sulfites on their labels. The FDA estimates that one in 100 people is sulfite-sensitive to some degree, but for the 10 percent of the population who are asthmatic, up to 5 percent are at risk of having an adverse reaction to the substance.

Current methods of analysis of sulfite include basic test strips, an iodide-iodate titration,[1] and sulfite acidification to sulfur dioxide gas. These kits vary in price and accuracy. However, a spectroscopic method would be cheaper because it would only require a spectrophotometer, which is already available in most scientific labs. Further, it would be convenient, reliable, and accurate at low concentrations. The aim of the research was to determine a simple spectroscopic method to measure sulfite in solution using 5-5¢-Dithiobis-(2-nitrobenzoic acid), or DTNB (also called Ellman's reagent). The method established in the research is also compared to previous studies on DTNB.

Since the first synthesis of 5-5¢-Dithiobis-(2-nitrobenzoic acid), DTNB, by George L. Ellman in 1959 for the quantitative analysis of mercaptans, "Ellman's reagent" has been commonly used for the determination of thiols in biochemical samples.[2] An organic disulfide, DTNB reacts with aliphatic thiol compounds to produce equimolar concentrations of a mixed disulfide and a luminescent thiol, 5-mercapto-2-nitrobenzoate (MNB), according to Equation 1.

R¢SSR¢ + RSH fi R¢SSR + R¢SH (1)

The use of DTNB has been extended to the reaction with sulfite ions (SO_3^{2-}), which displaces the thiol anion forming an organic thiosulfate, called a "Bunte" salt (Equation 2).[3,4,5,6]

$RSSR + SO_3^{2-}$ fi $RSSO_3^- + RS^-$ (2)

The reaction of DTNB with sulfite ions has led to studies for a spectrophotometric determination of sulfite.[3,4] While useful in biochemical samples, the method for sulfite analysis proposed by Johnston et. al. may not be effective in aqueous solutions. Such a method may interest the boiler industry. Humphrey et. al. demonstrated the usefulness of a similar method in aqueous solutions stabilized with the disodium salt of ethylenediamine tetraacetate (EDTA). This compound was introduced with the intention of stabilizing the readily oxidizable sulfite in aerated solutions. The method presented here demonstrates an effective sulfite assay in aqueous solutions without the use of EDTA and outlines some of the parameters governing the reaction of DTNB with sulfite and with L-cysteine in the presence of oxygen and ultraviolet light. A detailed comparison of the present method with a commercially available titration kit demonstrates the enhanced accuracy (<2% avg.

relative error) and range of analysis (<1ppm).

Experimental

Reagents. Ellman's reagent, DTNB (99%), was obtained from Aldrich Chemical Co. in Milwaukee, Wis.; laboratory-grade sodium sulfite was obtained from Flinn Scientific, Inc., in Batavia, Ill.; L-cysteine (98+%) and Tris-[hydroxymethyl]-aminomethane (99.9+%) were obtained from Sigma Chemical Co. in St. Louis, Mo. These reagents were used without further purification.

Solutions. The DTNB solutions were prepared 1.0 x 10^{-3}M by dissolving the DTNB with about 1mL 95% ethanol and diluting with N_2-purged pH 8.0 0.1M Tris buffer. Further dilutions yielded 1.0 x 10^{-4}M and 5.0 x 10^{-5}M DTNB solutions. The sodium sulfite solutions were prepared in N_2-purged water and diluted in volumetric flasks to obtain 10^{-4}M samples. Aqueous 2.5 x 10^{-4}M L-cysteine solutions were similarly prepared.

Equipment. Absorption measurements were made with a Perkin Ellmer Model Lambda 3B UV-visible spectrophotometer, using quartz cuvettes with 1cm path lengths. Comparison testing was done with a Hach Inc. titration analysis 1-200mg/L sulfite detection kit (Model SU-5, Cat. #1480-02).

Procedure. For the reaction of DTNB and cysteine, the cysteine was added in fivefold excess relative to DTNB to ensure complete dissociation of MNB. The cysteine was directly introduced into the solutions. The concentration of DTNB was made in fivefold excess relative to the concentration of the sulfite solutions. The DTNB and sulfite were reacted in equal volumes of 5mL, and all absorbance measurements were taken from about 2mL aliquots in triplicate. Reference solutions included a 50:50 mixture of DTNB in pH 8 buffer solution and deionized water.

Results and Discussion

DTNB-Cysteine reaction. In order to determine the em of MNB, a standard reaction was followed with DTNB and L-cysteine. It was observed that the cysteine completely cleaved the DTNB and formed double the molarity of the chromophoric thiol (MNB). Note that this is contrary to Equation 1; the cysteine is oxidized and forms cystine, while the DTNB is reduced and cleaved at the disulfide bond. Using a spectrophotometer, the peak absorbance at 410nm of 10^{-4}M MNB was determined to be 1.660 with a molar extinction coefficient of 16,600$cm^{-1}M^{-1}$. This value does not equal the em previously reported.[1,4,7] Humphrey et. al. documented 15,500$cm^{-1}M^{-1}$ as the em value of MNB. Discrepancies in the em values of MNB are also noted by Riddles et. al.[8]

DTNB-Sulfite reaction. It was hypothesized that a solution of 1.0 x 10^{-4}M sulfite would react similarly with DTNB to form 1.0 x 10^{-4}M MNB (see Figure 1). This is in accordance with Equation 2; the sulfite displaces the chromophoric thiol, MNB. The absorbance measurements of this solution at 410nm were near 1.600. This value is within 4% of the absorbance from the DTNB-cysteine reaction, justifying the reaction of DTNB with sulfite as seen in Equation 2. The absorbance of MNB was then measured as a function of the initial sulfite concentration for various samples. The data demonstrated a linear relationship between the absorbance of MNB and initial concentration of sulfite (see Tables 1 and 2). The sensitivity of this method has been demonstrated as low as 10^{-5}M sulfite.

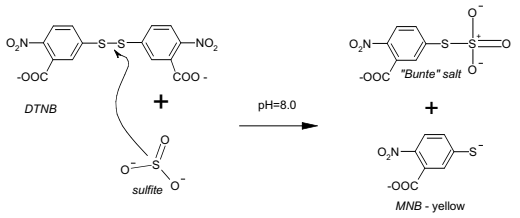

Figure 1. MNB (5-mercapto-2-nitrobenzoate) is displaced by sulfite.

mL MNB	mL SO_3^{2-}	mL Tris Buffer	[SO_3^{2-}] (mol/L)	Avg. Abs.
5	5	0	1.0x10^{-4}	1.610
5	4	1	8.0x10^{-5}	1.300
5	3	2	6.0x10^{-5}	1.008
5	2	3	4.0x10^{-5}	0.718
5	1	4	2.0x10^{-5}	0.434

Table 1. Comparison of the concentration of sulfite with the absorbance of MNB produced in the DTNB-sulfite reaction.

mL MNB	mL SO_3^{2-}	mL Tris Buffer	[SO_3^{2-}] (mol/L)	Avg. Abs.
5	5	0	$1.0x10^{-4}$	1.520
5	4	1	$8.0x10^{-5}$	1.230
5	3	2	$6.0x10^{-5}$	0.940
5	2	3	$4.0x10^{-5}$	0.637
5	1	4	$2.0x10^{-5}$	0.335
10	1	9	$1.0x10^{-5}$	0.186

Table 2. Comparison of the concentration of sulfite with the absorbance of MNB produced in the DTNB-sulfite reaction with adjusted spectroscopic reference solution.

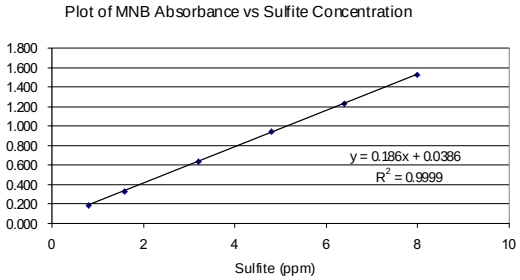

Figure 2. Adjusted sulfite determination graph.

Sulfite sample	Actual Mass (g)	Calculated Mass (g)	Error
A	.1261	.1298	2.90%
B	.0946	.0966	0.06%
C	.0631	.0656	4.00%
D	.0315	.0318	0.83%
Average % error	—	—	1.95%

Table 3. Calculated percent error from the DTNB-sulfite method.

Standard sample, Sulfite (ppm)	Hach Inc. kit (ppm)	Error
16	14.7	8.0%
12.8	11.5	10.0%
9.6	8.3	13.3%
6.4	5.8	10.0%
1.6	1.9	20.0%
Average % error	—	12.3%

Table 4. Calculated percent error for the Hach Inc. detector at low sulfite concentrations.

Validation. This method was validated with several standard sulfite samples and determined to be accurate to within a 2% average relative error (see Table 3) for dilute concentrations of sulfite (10^{-5} – 10^{-4} M). The method was then tested against the accuracy of a commercial sulfite detection kit. The Hach Inc. titration analysis sulfite detection kit had an average relative error of 12% (see Table 4) for dilute sulfite solutions.

Stability of Sulfite Solutions. It was initially observed that the absorbance of MNB was less than expected and decreased over time after the DTNB-sulfite reaction. The presence of oxygen was found responsible for the rapid degradation of sulfite solutions, and all dilutions were then done with nitrogen-purged water. This differs from the EDTA in pH 7 buffer shown in the results of Humphrey et. al. Johnston et. al. similarly conducted their experimentation in pH 7 buffer, but neglected to indicate sulfite degradation. Nevertheless, there is still uncertainty over the time dependency of the nitrogen-purged DTNB-sulfite reaction. The absorbance of MNB still decreased significantly after a period of about 20 minutes. There is a possibility that UV light will degrade MNB or promote a side reaction. In a DTNB-sulfite reaction placed in separate cuvettes, one in the dark, and one in UV light, MNB in the cuvette in the presence of UV light had a significantly lower absorbance. Nevertheless, all reactions were conducted expeditiously.

Application. Other existing techniques for sulfite analysis are either very expensive (such as analyzing the SO_2 gas produced under acid treatment) or have limited accuracy and utility (such as iodine-iodate titration). The present technique, however, could be translated into a simple color chart comparison for field use. One drawback to the use of DTNB is that it will react with any other compounds present that contain thiol groups; yet, in some interesting cases such as in the water boiler industry, other such compounds are absent, and this technique would provide a reliable, inexpensive, and field-usable method for determining sulfite concentration.

Boiler water is normally basic (pH 8–11), and the added sulfite (20–80ppm) and phosphate (30–80ppm) prevent scaling or corrosion on the walls of the boiler tanks. The reducing agent, sulfite, acts as an oxygen scavenger, forming the sulfate ion and preventing electrochemical side reactions.

Boiler water also does not contain any other compounds that may skew the results. The method was used to test the boiler water with sufficient accuracy. Methods employed were the filtration of the boiler water sediment and dilution (by a factor of 10) to bring the sulfite levels in the desired range of 0.8–8ppm. The results of the DTNB-sulfite method were corroborated with the Hach Inc. detector kit, which is accurate at higher sulfite concentrations. This showed that the DTNB-sulfite method is applicable and advantageous in the water boiler industry. The method is currently being researched for use in other industries, such as food and wine.

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Detection of Cotinine in Blood Plasma by HPLC MS/MS

Oneil Bhalala

Abstract

Tobacco smoking is a major killer in the United States and is attributed to approximately 434,000 deaths per year. Primary and secondary exposure to tobacco and tobacco smoke can be monitored by measuring cotinine levels in blood, urine, as well as other matrices. This article describes a HPLC MS/MS assay to detect low concentration levels of cotinine in blood plasma. The assay was developed at Children's Hospital, Boston, and thus it was specifically designed for use with young children. This assay allows for high throughput and turnaround because it does not use a column-based purification process; it is also fairly inexpensive, using common laboratory reagents. Upon completion of the study, the concentration ranges were found to be accurate from 0.l to10.0 ng/mL. The limit of quantitation was calculated to be 0.2 ng/mL (CV% < 20%, accuracy range ± 20%). The HPLC MS/MS assay is now ready for comparison tests with the ELISA test using patient plasma samples.

Introduction

About one in five U.S. deaths are a result of tobacco usage.[1] It is one of the main causes of premature death in the country. Tobacco use, mainly in the form of cigarette smoking, has been shown to increase the chances of various types of mouth and lung cancers, cardiovascular disease, and emphysema.

The harmful effects of tobacco can be seen in both active and passive smokers. Active smokers are those who smoke tobacco and tobacco products, while passive smokers are those exposed to tobacco smoke. Exposure of nonsmokers includes second-hand smoke from the air, as well as fetus exposure due to maternal smoking. Passive tobacco exposure is a major health problem in the United States, and is classified as a Group A carcinogen by the Environmental Protection Agency. Due to the magnitude of this health problem, analysis of passive tobacco smoke has increased over the years.

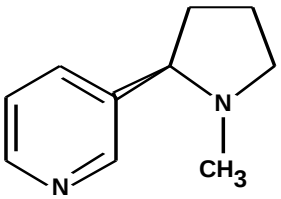

Figure 1. Chemical structure of nicotine.

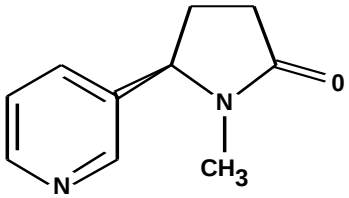

Figure 2. Chemical structure of cotinine.

Studies have shown that nicotine and cotinine are present in both active and passive smokers alike. Nicotine (Figure 1) is a natural product of tobacco, and it is present in the body with a half-life of 1–2 hours. Cotinine (Figure 2), on the other hand, is a metabolite of nicotine with a half-life of 18–20 hours.[2] The longer half-life makes cotinine a more stable and prominent compound in the human body than nicotine. This makes it a more desirable compound, as it is easier to analyze. The objective of this study was to develop an assay to detect and quantify cotinine levels in blood plasma.

The primary patient population of Children's Hospital, Boston, consists of adolescents and young teenagers. Given this patient demographic, any samples that the hospital receives would be from passive smokers. Therefore, the cotinine levels in their blood (<10 ng/mL) would be much lower than in active smokers (≥10 – 15 ng/mL).[3] Previous cotinine assays were not sufficient because the extraction procedures were too long and complicated, the limits of quantitation were too high, or the sample matrix was incompatible.[4] An inexpensive assay with a high throughput, a low limit of quantitation, and plasma as the sample matrix was designed to meet these needs.

Doctors at the hospital could analyze patients' cotinine levels quickly and accurately using the designed assay. By having the results back within a few working days, doctors could then promptly tailor treatments to the patient's needs and condition. In addition, this assay greatly enhances the ability to detect cotinine levels in young children and teenagers.

Method

A high-throughput, inexpensive, and accurate assay was developed to meet the needs of Children's Hospital, Boston. The assay utilized the PE SCIEX API 3000 with the TubroIon Spray HPLC MS/MS (high-performance liquid chromatography tandem mass spectrometer) machine owned by the hospital to detect the cotinine levels in blood plasma. Before the sample could be injected into the HPLC MS/MS, the cotinine was first purified and separated from the rest of the plasma. The assay was divided into the following five steps: sample acquisition, standards creation, sample protein precipitation, sample reconstitution, and HPLC MS/MS configuration and injection.

Sample Acquisition

A blood sample was acquired from one of the workers in the Hematology Lab at Children's Hospital, Boston. The blood sample was centrifuged at 14,000 rpms for 10 minutes to separate out the plasma. The plasma was then transferred to a clean tube and stored at -20° C for preservation. Before each use, the plasma sample was thawed at room temperature and certain amounts were transferred to other tubes used for the experiment. The remainder of the sample was placed back into a -20° C freezer for storage.

Standards Creation

Blood plasma samples were equilibrated with a D_3 – cotinine Internal Standard (ISTD). 25 µL of plasma of various cotinine concentrations were mixed with 25 µL of ISTD in 1.5mL Eppendorf tubes. The following are the cotinine concentrations that were used: 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 5.0, and 10.0 ng/mL. These standards were used to measure assay and machine accuracy and recovery during HPLC MS/MS analysis.

Sample Protein Precipitation

500 µL of Acetonitrile was mixed into each tube to precipitate out heavy proteins. The mixtures were vortexed for 30 seconds

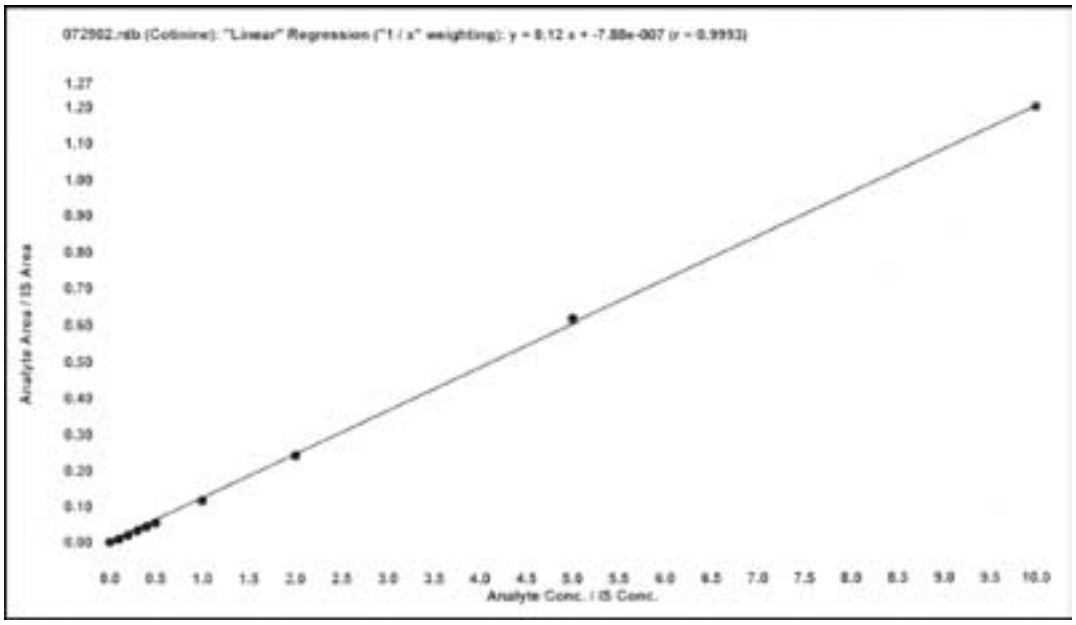

Figure 3. Calibration curve from 7-29-02. R-Value = 0.9993.

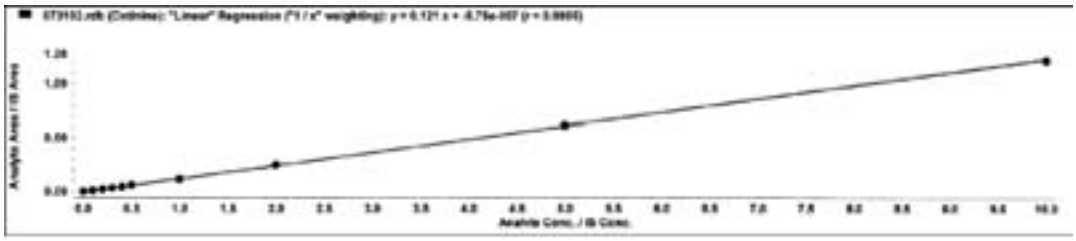

Figure 4. Calibration curve from 7-31-02. R-Value = 0.9995.

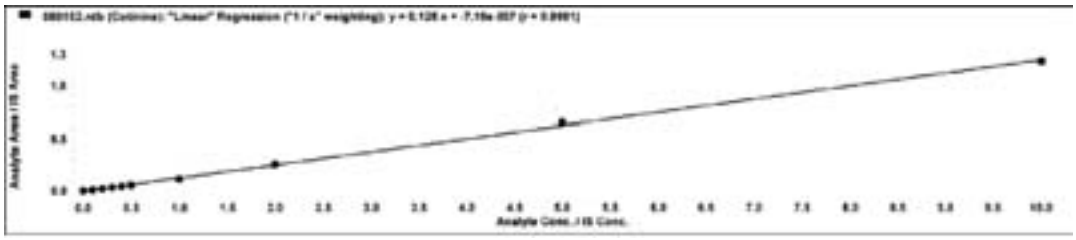

Figure 5. Calibration curve from 8-1-02. R-Value = 0.9991.

Figures 3 to 5 show the calibration curves from the three analysis days. As demonstrated, all three curves were linear from 0.0 to 10.0 ng/mL of cotinine concentration. This range covered both the high and low ends of cotinine concentrations. More importantly, the R-value from each of the days was greater than 0.9990, as desired.

and then centrifuged for 4 minutes at 14,000 rpms in order to form a cell debris pellet. 500 µL of the supernatant was then placed in clean glass test tubes and placed in a N_2 vaporizer for 15 minutes to evaporate the liquid from the supernatant.

Sample Reconstitution

Each glass test tube was reconstituted with 150 µL of MeOH to dissolve the cotinine and any remaining proteins. The tubes were again vortexed for 30 seconds to further precipitate out any remaining heavy proteins. The solution in each tube was transferred to 150 µL vials for HPLC MS/MS analysis.

HPLC MS/MS Configuration and Injection

The buffer solution used for the assay was 10 mM Ammonium Acetate in 30% MeOH.

20 µL of each sample was injected into the HPLC MS/MS at a flow rate of 200 µL/min. The analysis time was set for 2 minutes. The temperature was set to 450° C. The Q1/Q3 ratio for cotinine is 177/80, and the ratio for D_3 – cotinine is 180/80.

Results

Samples were prepared on 7-29-02 and analyzed on 7-29-02, 7-31-02, and 8-1-02 for accuracy and inter- and intra-day precision. A set of samples was created for intra-day analysis and another set was created for inter-day analysis. This enabled us to test for HPLC MS/MS accuracy as well as inter- and intra-day precision. The information obtained from these analyses helped determine the detection and quantification abilities of the newly developed assay.

Calibration Curve

Standard samples were prepared with cotinine concentrations of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 5.0, and 10.0 ng/mL. These concentrations allowed us to test the accuracy and precision of both the high-end concentrations (5.0 – 10.0 ng/mL) and the low-end concentrations (0.1 – 0.5 ng/mL) as well as to identify the limit of quantitation of the assay. Each sample also contained 10.0 ng/mL of ISTD (D_3 – cotinine). This internal standard aided in determining the accuracy of both the assay and the machine.

The data from the standard samples was used to construct a calibration curve for each testing day. The calibration curve was linear, with a "1/x" weighting. A regression value (R-value) greater than R = 0.9990 was desired. Figures 3 to 5 show the calibration curves from the three days of analysis.

Inter-Day Precision

One sample each of 0.5 and 2.0 ng/mL cotinine containing 10.0 ng/mL of ISTD was prepared and run three times each in the HPLC MS/MS. The data was used to calculate the standard deviation, mean, and correlation value (CV%). The CV% was calculated by the quotient of the standard deviation and mean. A CV% of less than 20% was desired. Table 1 contains the inter-day precision data from the two types of samples.

Table 1. Inter-Day precision of 0.5 and 2.0 ng/mL of cotinine. Table 1 provides the overall values obtained from the inter-day analysis of 0.5 and 2.0 ng/mL of cotinine. As shown, the CV% is well below the 20% maximum for both samples. The 0.5 ng/mL sample has a CV% of 3.75, while the 2.0 ng/mL sample has a CV% of 11.35%.

Sample	0.5 ng/mL	2.0 ng/mL
S.D.	4.93E+05	1.25E+06
Mean	1.32E+07	1.10E+07
N	3	3
CV%	3.75	11.35

Intra-Day Precision

Samples were prepared with 0.5 and 2.0 ng/mL of cotinine containing 10.0 ng/mL ISTD. These samples were run on 7-29-02, 7-31-02, and 8-01-02 to determine the intra-day precision. The standard deviation, mean, and CV% were calculated from the data. Again, a CV% of less than 20% was desired. Table 2 contains the intra-day precision data for the two types of samples.

Accuracy

The recovery rate for the 0.5 and 2.0 ng/mL was calculated by taking the quotient of the measured cotinine concentration and prepared cotinine concentration for each of the samples. The desired recovery range was between 80% and 120%. Samples between this recovery range were deemed accurate. Table 3 provides the recovery range for each of the samples.

Limit of Quantitation

The limit of quantitation is determined as the lowest concentration of cotinine that has a CV% of less than 20% as well as a recovery rate between 80% and 120%. The analysis showed that 0.2 ng/mL of cotinine was the limit of quantitation for this assay because it met these criteria. The only lower concentration, 0.1 ng/mL, did not meet these criteria.

Discussion

The goal of this project was to develop an assay that would detect and quantify the levels of cotinine in blood plasma. Given that the assay would be used at Childern's Hospital, Boston, it also needed to be sensitive—have a low limit of quantitation as well as high throughput capabilities. We believe that an adequate assay was developed.

The developed assay uses a HPLC MS/MS without the need of a column to purify the sample. This significantly reduces the time needed per sample. If a column was to be used, the run time for each of the patient samples would easily triple or quadruple, because the sample would enter and exit the column and the column would need cleaning between sample runs. Previous assays performed in the laboratory demonstrated that a column did not improve the resolution or sensitivity of the assay. Therefore, a column was not included as part of the proposed assay.

In addition, blood plasma is the most compatible matrix for the target patients (young children and teenagers) at the hospital; it is very hard to obtain enough saliva or urine samples from infants and toddlers. Also, this assay requires the use of only 25 µL of plasma, thus only a small amount of blood needs to be drawn from the patient.

The sample matrix also needs to be purified before it can enter the HPLC MS/MS. The extraction method that was developed for the assay is sufficient in its purification abilities. It precipitates out the heavy proteins with the use of Acetonitrile, a common and easily obtainable laboratory reagent. The other steps in the extraction process further purify the sample, such as the N_2 evaporation. This assay does not damage the HPLC MS/MS machine by injecting unpurified samples, thereby safely allowing for repeated analyses.

The results showed the assay's limits of quantitative capabilities. An upper bound of 10.0 ng/mL and a lower bound of 0.2 ng/mL of cotinine were established. These were the upper and lower limits in which the assay would quantitate effectively; any concentration outside of this range will produce unreliable results using the developed assay. Because the hospital deals primarily with children, a modified upper bound of 2.0 ng/mL was created—only significant passive smokers contain over 2.0 ng/mL of cotinine in their blood, as demonstrated by previous studies. An example of a significant passive smoker is one who is constantly exposed to environmental smoke, such as a waitress. The hospital is mainly concerned with relatively low passive smokers who have plasma cotinine levels much lower than 2.0 ng/mL.

Table 2. Intra-Day precision of 0.5 and 2.0 ng/mL of cotinine. Table 2 provides the overall values obtained from the intra-day analysis of 0.5 and 2.0 ng/mL of cotinine. As shown, the CV% values for each of the samples were under the maximum level of 20%. These matched the desired results for this analysis.

Sample	0.5 ng/mL			2.0 ng/mL		
S.D	1.04E+05	1.62E+04	6.64E+05	1.05E+05	2.53E+05	5.07E+05
Mean	5.49E+05	1.92E+05	4.77E+06	2.59E+06	2.47E+06	4.81E+06
N	7	9	9	7	9	9
CV %	18.94	8.42	13.91	4.05	10.25	10.54

Table 3. Concentration of cotinine and recovery rate for 0.5 and 2.0 ng/mL of cotinine. Table 3 shows the recovery rates of the 36 analyzed samples. As shown in the table, all 18 of the 0.5 ng/mL cotinine samples had a recovery rate between 80% and 120%. Based on the criterion established before, these 18 compounds were determined to be accurate. For 2.0 ng/mL cotinine samples, 15 of the 18 compounds had a recovery rate between 80% and 120%. Therefore, 15 of 18 2.0 ng/mL cotinine samples were accurate.

0.5 ng/mL (N=18)				2.0 ng/mL (N=18)			
Conc.	Recovery	Conc.	Recovery	Conc.	Recovery	Conc.	Recovery
0.464	92.80	0.425	85.00	1.410	70.50	1.870	93.50
0.456	91.20	0.433	86.60	1.470	73.50	1.920	96.00
0.468	93.60	0.438	87.60	1.430	71.50	1.900	95.00
0.505	101.00	0.493	98.60	1.870	93.50	2.030	101.50
0.492	98.40	0.489	97.80	1.850	92.50	2.050	102.50
0.509	101.80	0.487	97.40	1.810	90.50	2.140	107.00
0.493	98.60	0.477	95.40	1.680	84.00	2.030	101.50
0.470	94.00	0.483	96.60	1.700	85.00	2.040	102.00
0.491	98.20	0.492	98.40	1.750	87.50	2.110	105.50

It is the hospital's goal to identify those patients who have a low, yet significant, exposure to environmental smoke. This assay allows the hospital to do exactly that.

The experimental data showed that the samples were linear within the range of 0.2 to 2.0 ng/mL of cotinine. This is substantiated by the R-value of the calibration curve being greater than 0.9990 and the CV% being less than 20%. From the data, we conclude that our assay is also accurate from 0.2 to 2.0 ng/mL.

The data gathered from the analyses indicates that we accomplished our goal of developing an assay that is inexpensive, sensitive (low limit of quantitation), and has high throughput capabilities.

However, before this can be used as a standard patient assay in the hospital, a comparison test needs to be carried out. First, samples need to be collected from hospital patients. The cotinine concentrations should then be quantitated using the developed assay and the ELISA test, the standard assay currently used. If this comparison shows that the newly developed assay is just as accurate as the ELISA test, then the hospital will be able to quantify cotinine levels in patient samples using the new assay. This study supports the outcome that the developed assay will be as accurate as the ELISA test.

Acknowledgments

I would like to thank Dr. Nader Rifai, director of Clinical Chemistry at Children's Hospital, Boston, who allowed me to spearhead the project. I would also like to acknowledge the help of Charlie Bu, Terry Law, and Masa Sakamuto as well as Dr. Neal Lerner for his help in editing this paper.

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Probing Solar Panel Design Systems

Alexander C. De Feo

Current drawbacks of utilizing solar energy are high initial costs and the inconvenience of high maintenance. Although the use of solar energy has been steadily growing, it is only 0.08 percent of the energy-producing market within the United States.[1] One of the growing areas in solar research has been the mounting brackets of solar arrays. The goal of this design project is to develop innovative methods for producing an inexpensive mounting system to reduce initial costs of a photovoltaic solar grid. Limited engineering research in this area has kept these panels retailing at the average cost of $600 per panel, while the mounting system costs per panel are over $100. Thus, this article will discuss possible cost-effective solutions for mounting systems. In addition, experimental methods for testing the panels will be suggested.

"Solar energy has an untapped potential ...current efficiency conversion from sunrays to electricity is only 12%"

After reviewing current designs available on the market, an improved design was established by following these specific functionality requirements: (1) a mounting system to install on commercial and industrial flat roofs; (2) a simple and flexible design to facilitate mass production; (3) injected molded recycled plastic rather than traditional steel structures, to reduce corrosion on the system; (4) a simple and quick installation method such that individual panels can be easily removed; (5) a freestanding system that eliminates the need to penetrate roof seals; and (6) a wind rating to conform to building code specifications.

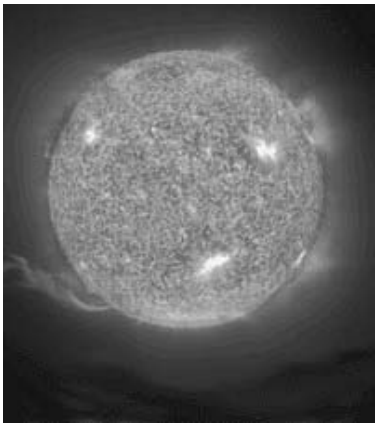

Image courtesy of SOHO/EIT consortium. SOHO is a project of international cooperation between ESA and NASA.

The following sections will discuss four possible designs of solar mounts for different applications. The Modular design meets all of the above functionality requirements and is intended for heavy winds. The second design, Corners, is more flexible; however, it will not protect the panels under heavy loads. For greater solar conversion efficiency, the Tilt design yields the best results. The last design, Awnings, aims to be visually exposed and uses novel materials.

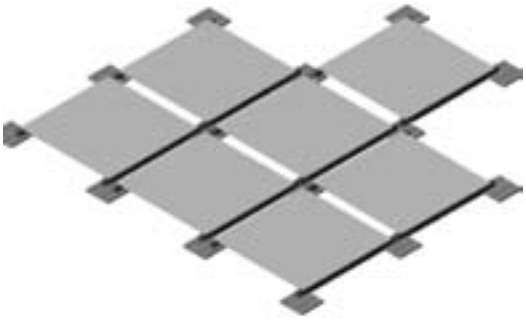

Figure 1. Modular array of seven panels.

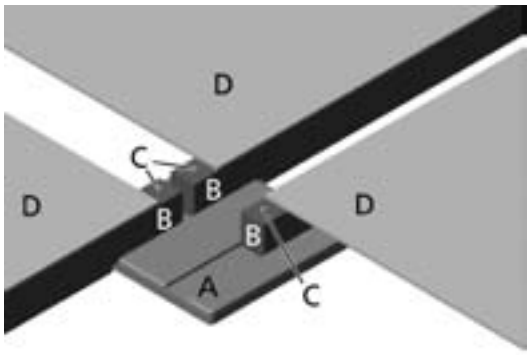

Figure 2. Parts identification of foot (A), slats (B), bolts (C), and panels (D).

Design I — Modular

The initial design is simple yet robust, and is composed of only two elements, a foot and slat. As shown in Figure 1, the panels are mounted to slats that are joined by feet. The grooves in the foot component shown in Figure 2 constrain the slats from rotation. A single stainless steel bolt constrains the slat in the vertical as well as lateral axes.

As with traditional mounting systems, the panels are glued to the slats. The installation costs are greatly reduced because there is no on-site customization for oddly shaped roofs; that is, the panels can be arranged in several configurations to adapt to roof obstructions. When mass-produced, the foot and slat components cost less then $3 each, and the total cost per panel is about $18. For smaller-scale production runs, a wood/plastic composite lumber could be used as an alternate material to injected molded plastic. This composite material is corrosive-resistant and can be cut and drilled similarly to wood.

This design meets all of the functionality requirements named previously, except for the 90 mph wind rating of Massachusetts building codes, which has yet to be proven. Due to the complications in theoretically modeling the system, an experimental model has to be made of air flowing over the top of commercial buildings, and the lift and drag forces on the system must be measured. Because the weight of the panels is not sufficient for keeping the system stationary, the corners of the array have to be constrained by cable stays that can be mounted to the side walls of the roof. The aims of this experimental test are to determine the necessary constraining forces as well as to find any system complications such as resonance frequencies that may arise in storm conditions. Once the forces are calculated, finite element analysis software can be used to specify the contact stresses in the glass panels.

The high wind-speed conditions on the roof of a commercial building will be simulated at the MIT water tunnel. The schematic in Figure 3 shows a delrin structure simulating the flow of air over a commercial building. The tunnel is 1.2 meters long and has a cross section of 0.5m x 0.5m. The water flows over the bulkhead to simulate the walls around the roof of a commercial building. The one-third-scale panel in the experiment is a stainless steel plate of dimensions 0.64m x 0.32m x 0.008m. As the water flows over the bulkhead, water can flow above and below the panel because there is a clearance of 0.01m to simulate ventilation under a panel. To vary the position of the panel, the distance between the

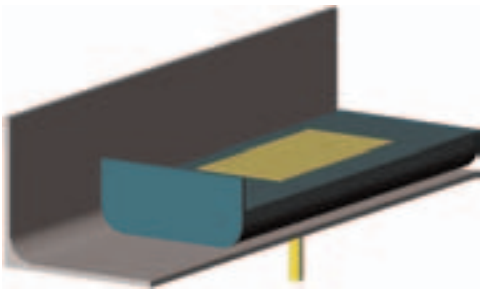

Figure 3. Experimental schematic cutaway of tunnel section. The water flows from left to right at a maximum velocity of 5m/s. The distance between the panel and bulkhead can be adjusted up to 0.64 meters.

panel and bulkhead can be adjusted up to 0.64 meters.

Using nondimensional analysis, the medium and scaling properties can be simulated for the actual conditions. The Reynolds number in Equation 1 can be used to equate the actual and simulated conditions.

$$\mathrm{Re} = \frac{\rho \cdot v \cdot L}{\mu}$$

Equation 1. Reynolds number, where ρ is the density, v is the velocity, L is the width of the panel, and μ is the viscosity.

The forces on the panel will be measured using a six-degree-of-freedom dynometer that is positioned below the water tunnel. The measured vertical and horizontal forces can be scaled appropriately by using the coefficient of lift and drag in Equations 2 and 3, respectively:

$$C_L = \frac{F_L}{0.5 \cdot \rho \cdot v^2 \cdot A}$$

$$C_D = \frac{F_D}{0.5 \cdot \rho \cdot v^2 \cdot A}$$

Equations 2 and 3. Lift and drag coefficients, where F_L is the vertical force, F_D is the horizontal force, v is the velocity, and A is the area of the panel perpendicular to the force.

Design II — Corners

For every design there are revisions for different applications. In this case, the customer has dictated the creation of the three subsequent designs. MIT facilities recently received a grant to install solar panels on campus.[2] The supplier of solar panels uses various panel sizes that are not compatible with the Modular design. It would therefore be necessary to have shorter or longer slats depending on the specific size of the panel as there is not one industry standard. This issue spurred the idea of having a configuration that is not constrained by the specific dimensions of the panel. Removing the slats and modifying the foot component developed a design whereby the panel is only constrained in the corners. As shown in Figure 4, the corners of the panel are sandwiched in between two symmetric parts.

Figure 4. Depiction of bottom corner component with panel. The corresponding symmetrical corner component is not shown.

Figure 5. Array of panels with corner design depicting partial installation.

To reduce possible concentrated stresses on the extremely inelastic glass panels, a thin layer of neoprene can be inserted between the plastic and glass. The installation efforts are also reduced with this design because the gluing phase is eliminated. The drawback is that a small portion of the photovoltaic cells are blocked off from sunlight. In addition, the system is not as robust as the first: The slat backing does not fully support the panels. Under high winds, vibrations may dangerously strain the panels, reducing the life of the photovoltaic cells.

Design III — Tilt

These first two designs were economically driven, streamlined systems. This next design aims to variably tilt the panels to "follow the sun," which can increase photovoltaic efficiency up to 40 percent. As the sun travels from east to west, tilting the panels in one axis will increase efficiency by 20 percent. In the northeast region of the United States, the sun also moves north and south, yielding another 20 percent increase in efficiency if the panels are tilted in a

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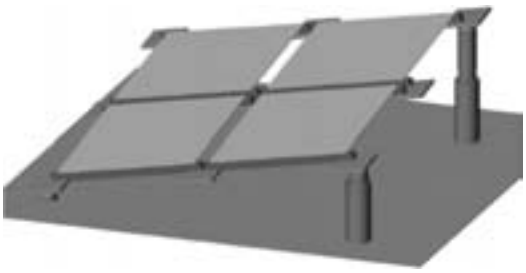

Figure 6. Schematic of a four-panel tilted array. Pneumatic actuators are positioned in two of the corners (right side).

second axis. The modular design with slats can be altered by adding two moving actuators in adjacent corners of a square array. These actuators could be comprised of either a lead screw configuration or an air piston driven by an air compressor, depicted in Figure 6.

The tracking control system could be composed of either an optical tracker or a scheduled position based on the given day of the year. Because these panels are elevated, there are additional bending stresses due to the weight of the panels. This will dictate brackets in addition to the foot components or even slats that extend the length of the array. The limiting factor of this more glamorous design is based on the added fixed cost of the movable parts as well as the marginal costs of the added energy consumption.

Design IV — Awnings

Besides the economic barriers, solar energy awareness has been extremely limited. One of the aims of the MIT solar panel project is to make the panels visible to everyone on campus. Consequently, the first three designs are limited to isolated roofs. A fourth design would place the panels on the south sides of buildings to resemble awnings. The construction would be a wood/plastic composite lumber as supporting brackets, with alternative foot junctions.

Conclusion

The creation of better solar panel mounting systems has been a growing development in the solar energy industry because they provide electricity during peak midday usage and can be installed on small scales. This independence decreases the strain on

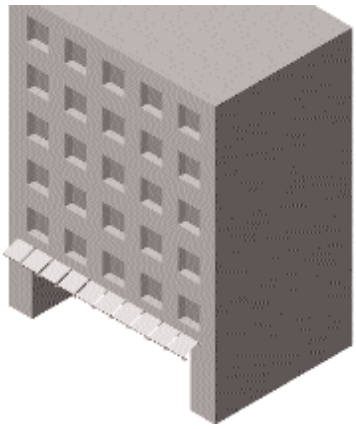

Figure 7. Awning mounting system installed on the south side of a concrete building for maximum sun exposure and visibility.

electricity grids and eliminates the energy loss of distributed energy.

The current efficiency conversion from sunrays to electricity is only 12 percent, and once more research is invested in development this efficiency can increase. If the initial cost of installing a solar panel array is decreased due to improved mounting systems, more solar packages will be purchased, increasing the market size. As the solar energy industry expands, more profits can be cycled back into R&D to increase the photovoltaic efficiencies. The Massachusetts Renewable Energy Trust has given MIT facilities a grant to install solar panels on and off campus to increase the efforts of promoting solar energy. Ultimately, producing a solar panel package that is competitive to fossil fuels is a long-term goal that can only be achieved once this novel technology becomes mass-produced.

Acknowledgments

I would like to thank Island Energy Solutions for the inspiration of improved solar panel mounting systems. The initial funding for this project was graciously provided by the Paul E. Gray (1954) Endowed Fund for UROP. Advising Professor Alexandra Techet and water tunnel engineer Richard Kimball gave me invaluable guidance. Laxmi Rao from MIT Facilities (Massachusetts Renewable Energy Trust) inspired this journal article.

Environmental Accounting:

Project Financing and Strategic Interactions

Angela S. Bassa

Abstract

This paper discusses the Kyoto Protocol propositions for reducing global warming by financing appropriate projects in developing countries through the Clean Development Mechanism. The paper shows that care must be taken to avoid the global loss of social well-being.

Introduction

The Kyoto Protocol, agreed upon in December 1997, sets legally binding limits on greenhouse-gas emissions for developed countries.[4] Each country must achieve at least a 5 percent reduction from 1990 levels between 2008 and 2012, a significant departure from current trends. The protocol indicates specific areas for action and sets up a number of mechanisms for international cooperation. One such mechanism is called the Clean Development Mechanism (CDM). The CDM is intended to attenuate the foreseen economic impact of an abrupt reduction in the levels of emissions and to encourage cost-efficiency by establishing an international market for the negotiation of Greenhouse-Gas Emissions (GGEs) between developed and developing countries.

The CDM, as proposed in the protocol, allows for transactions of Credits of Emissions Reductions (CERs). These credits result from exchanges between countries and may be used by developed countries to achieve their GGEs reduction quotas. A list of the developed countries that were original signatories and were part of the Kyoto Protocol's Annex-I can be found in the Appendix, together with their relative levels of GGEs in 1990.

The Kyoto Protocol foresees that developing countries will participate solely through the CDM. Because the greenhouse effect results largely from the emissions of the developed countries, there is an effort to guarantee that the economic growth of these nations will not be curtailed by restrictive environmental policies. Also, imposing environmental controls

that result in economic costs to the developing countries would be impractical without some type of compensation.

CERs may be bought by a developed country or by a firm with headquarters in a developed country. Given the large volume of greenhouse gases emitted by these countries (about 80 percent of the total), it is apparent that, economically, the greater beneficiaries of the CDM will be developing countries with industrial parks that are not subject to strong environmental legislation. These developing countries could generate great volumes of CERs competing directly in the International Trading Quotas market (ITQ), the future emissions quota international market. The great appeal of these CERs is that the costs of emissions reduction in developing countries, it is assumed, are substantially lower than in developed countries.

Intuitively, the primary way in which these exchanges take place may seem to be via direct CER trading. However, direct CER trading with developing countries is not the only way that CERs could be passed onto developed countries. Firms located in developed countries may also finance their emissions-reduction projects directly, instead of trading quotas in an international market. The economies of the developing countries involved would have subsequent advantages from directly participating in a partnership project with a foreign firm from a developed country. Firstly, capital and technology would be attracted and would finance part of the development cost. Moreover, the projects would lead to an improvement in the environment of the affected areas. By taking into consideration the nature of developing economies and the large volume of CERs possibly demanded by developed countries, the question of who finances the projects for emissions reduction becomes fundamental. Another aspect to consider is that when firms from developing countries come to finance their own projects of emissions reduction to get CERs and offer them for sale, the developing economies are the ones financing the environmental policies in developed countries. This financing is amortized by the payment of the CERs.

There may be additional costs imposed on firms in developed countries for implementing this alternative solution. Contrary to initial expectations, as Tietenberg points out, it is possible that the firms headquartered in developing countries have no interest in allowing their emissions to be reduced (even without cost), because these firms expect their market share and their profits to increase due to the change in cost structure of the other firms that reduce their emissions.[7]

This paper develops a model in which such a result *always* emerges, and it does so as a consequence of definitive strategies of two firms: Firm 1 and Firm 2. Firm 1 is located in a developed country, and may or may not be interested in developing a project through the CDM. Firm 2 is located in a developing country which may or may not accept this partnership. The paper also develops a means of incentive in which the firm that proposes a project may induce a better situation for itself, affecting the decision of the other firm. With such a mechanism, the proper agency of environmental control in a developed country can determine a priori whether or not the project can implemented.

In Section 2, a few possible hypotheses are presented and refuted; one hypothesis is accepted and carried out. In section 3, the accepted model is established and its assumptions explained. In Section 4, the effects of the model are analysed and presented and possible consequences evaluated. In Section 5, an alternative conjecture for future work is proposed; it takes into consideration the possibility of external resource financing.

Hypothesis

Consider an economy with two countries and two firms. Say Firm 1 is located in Country A, Firm 2 is located in Country B, and both firms are able to do business in the two markets. Country A is developed, whereas Country B is in development. The impact of the CDM on the strategies of the firms is considered here, where the chosen strategy defines the production level. Before the adoption of the CDM, both firms are supposed to be maximizing profits given the competitor's strategies. To simplify, assume

an economy in which information is full, perfect, and symmetrical—a reasonable and convenient assumption in the Internet age.

In order to study the impact of a joint project of emissions reduction on the strategies of the firms, consider the following situation. Given the necessity to control specific GGEs associated with the production of some good in Country A, the agency of environmental control in this country decides to transfer the reduction costs to Firm 1, which is responsible for the GGEs. Firm 1 is assumed to be using the best possible technology for reducing emissions. Two possibilities exist: The agency charges a tax on Firm 1 to finance the purchase of quotas in the ITQ market; or Firm 1 develops a project in partnership with Firm 2, in Country B, that is not using the best technology available for emissions reduction. For simplicity, it is assumed that any project being considered for implementation will generate the exact amount of credits necessary to meet the expected reductions in Country A.

One hypothesis is that the market structure forces the production decisions to be taken in independent and simultaneous form, in a classic model of Cournot competition.[6] The existence of technological differences and input barriers cannot explain a market structure of this type when both firms directly compete in the markets of both countries. The only difference it allows between the firms is the cost due to pollution control. This counterexample can be found, for instance, in the paper and cellulose industry.[1]

Another hypothesis is that technology and plant scale are distinct in such a way that Firm 1 has a bigger plant and is more technologically intensive, with inferior marginal costs. In this way, Firm 1 could exert market pressure to determine, directly or indirectly, the prices of the good in the market. This situation is interpreted through the typical Stackelberg competition model[6] instead of a Cournot model.

This second hypothesis occurs even when the technologies are similar, as long as the production capacities of the firms are distinct. For instance, even though production costs are basically identical, one of the firms is able to raise offers in both markets in order to keep equilibrium marginal prices below production costs in a strategy of dumping. An example of these practices can frequently be found in the Chinese footwear industry, which counts on the state to finance its actions.[2]

This kind of situation is not an anomaly. Moreover, this is a limit-situation: One firm imposes leadership in the market due solely to its ability to use predatory behavior. However, strategies of dumping can become too costly, and their success depends on costs and profits determined in a monopolist market. Therefore, it is probable that firms that operate such strategies do not do so, although they do exert some sort of leadership in the market, either in price or quantity, as treated in this paper.

In general, leadership in the market is justified by scale economies or technological innovations. If such factors were incorporated into the hypotheses, then they would reduce the relative profits that Firm 2 would get once Firm 1, the leader, reduced its participation in the market due to the imposition of environmental policies in Country A. The model presented in Section 3 uses limit-situations where market leadership can be exerted. In these situations, an equilibrium that benefits both countries can emerge by considering the strategic interaction between the firms.

Model

Consider Firm 1 to be a leader company in a developed country, and Firm 2 a follower in a developing country. Their strategic interactions in the market are treated through a Stackelberg duopoly model.[6] The leader firm directly decides its profit maximization strategy by predicting the reaction function of the other firm. The latter, in turn, maximizes its profits, given the leader's chosen strategy.

Some assumptions are necessary so that the payoffs for both firms are determined:

1. The fixed costs for both firms are irreversible.[8]
2. The cost for Firm 1 to carry on the project in partnership with Firm 2 is also irreversible and equal to k.

3. The tax charged by the environmental agency is applied to the level of production of Firm 1 and is equal to δ, where $0 \leq \delta \leq 1$.
4. There is some type of restriction hindering the firms from establishing plants of production in the other country.
5. Both firms are profit maximizers.

Given the imposition of the emissions control agency, Firm 1 in Country A contemplates the following strategies: Either pay the agency tax or implement the project in partnership with Firm 2 in Country B. Firm 2, in turn, also contemplates two strategies: Accept or reject a partnership with Firm 1. Given Assumption 4 above, the possible strategies for Firm 1 are significantly reduced. In fact, as Motta and Thisse[5] indicate, the imposition of environmental standards in a country can lead to the relocation of the plants to countries where environmental policy practices are less restrictive. However, this situation only occurs in special circumstances, such as when the costs of implementing a new plant are relatively low in relation to the costs of the environmental policies. The capital-intensive nature of most firms makes this situation improbable.

For each country i, let Q_i be the total production, let P_i be the price established for the good being produced, and let α_i be the stand-alone consumption. According to Pindyck and Rubinfeld, the typical demand function is then:

We can assume that α_A is greater than α_B because consumption in developed Country A is likely to be larger than that of developing Country B. The costs of production are constant and equal to c for both firms.

The nature of a Stackelberg game is dynamic (Firm 2 plays after Firm 1), but the assumptions that the information is full, perfect, and symmetrical allow the payoff of each type of approach, by each firm, to be calculated. The game can then be reduced to its standard form, a reduced 2 x 2 payoff matrix, through a refinement known as retroactive induction. The payoff matrix of a two-firm game has rows labeled by Firm 1's strategies and columns labeled by Firm 2's strategies. The xyth entry of the matrix is the payoff that accrues to each firm in the event that the "row player" uses strategy x and the "column player" uses strategy y. When analyzing any game, we make the following assumptions about both firms:

1. Each firm makes the best possible move.
2. Each firm knows that its opponent is also making the best possible move.

The strategy for Firm j is an ordered pair of quantities (q_{jA}, q_{jB}) sold in Countries A and B, respectively. The reduced payoff matrix is given by the profit associated with the chosen strategies. The possible strategy pairs related to the environmental policies in Country A and the choice of Firm 2 regarding the implementation of an emissions reduction project are shown in Table 3-1.

Table 3-1. Dynamic Strategy Choices

Case 1	Firm 1 chooses to pay the tax Firm 2 would accept the project implementation
Case 2	Firm 1 chooses to pay the tax Firm 2 would not accept project implementation
Case 3	Firm 1 chooses to propose partnership Firm 2 does not accept project implementation
Case 4	Firm 1 chooses to propose partnership Firm 2 accepts project implementation

For comparison purposes, the equilibrium solutions are given here prior to the implementation of environmental policies. Since the algebra of the standard Stackelberg model is simple, only the strategies and the profits associated are presented. Additionally, the production cost c is such that the amounts of equilibrium are strictly positive.

Each firm presents the following profit functions π_i in their initial configurations:

(3-1)

(3-2)

Profits associated with equilibrium strategies π_i^* for each firm are given by

(3-3)

(3-4)

Quantities Q_i sold in each country i are given by

(3-5)

Case 1 in Table 3-1 occurs when Firm 1 prefers to pay the tax, even when Firm 2 would accept the implementation of a joint project of emissions reduction. In this case, the profits p_i are given by Equations 3-1a and 3-2a.

(3-1a)

(3-2a)

Recall that d is the tax charged by the environmental agency. Thus the updated profits for each firm associated with the equilibrium strategies p_i^* are given by expressions 3-3a and 3-4a.

(3-3a)

(3-4a)

Given that in Case 1 Firm 1 pays the tax d, the amounts negotiated in each country i are given by

(3-5a)

By comparing Equation 3-5 with Equation 3-5a, when Firm 1 opts to pay an environmental tax, the equilibrium quantities are reduced in both countries, and the prices must be raised relative to the original equilibrium. More importantly, Firm 1's profit may or may not decrease, while Firm 2's profit certainly increases.

Case 2 occurs when Firm 1 pays the tariff and Firm 2 does not accept project implementation. The strategies and payoffs associated with this case are the same as in Case 1. Therefore, when Firm 1 chooses to pay the tax, Firm 2's decisions do not affect the final result of the game.

Case 3 occurs when Firm 1 prefers to implement the project of emissions reduction, and Firm 2 accepts the partnership. Firm 1 then pays for all costs of the project k if there is a reduction of emissions in Country B. In this case, the profit functions are given by Equations 3-1b and 3-2b.

(3-1b)

(3-2b)

Because Firm 1 pays all the costs of the project, which are fixed and equal to k, the strategies and equilibrium payoffs do not differ from the initial situation, except in relation to the profit of Firm 1. Profits for each firm associated with the equilibrium strategies p_i^*, taking into account this cost, are given by Equations 3-3b and 3-4b.

(3-3b)

(3-4b)

It follows that Firm 1's choice modifies neither the prices nor the equilibrium quantities in either country.

Case 4 occurs when Firm 1 prefers to implement the project, but Firm 2 does not accept the partnership. In this case, Firm 2's

Table 3-2. Payoff Matrix

Case 1
Firm 1: Pays tax
Firm 2: Accepts project
Case 2
Firm 1: Pays tax
Firm 2: Refuses project
Case 3
Firm 1: Proposes project
Firm 2: Refuses project
Case 4
Firm 1: Proposes project
Firm 2: Accepts project

decision affects game payoffs because when it refuses the project, the best (and only) option for Firm 1 is to pay the tax. The strategies and equilibrium payoff in this case are identical to those indicated in Case 1.

Once payoffs for each case have been established, the payoff matrix can be expressed as shown in Table 3-2. Note that this matrix is formed using the assumption that the information is perfect, symmetrical, and full, and in this case, that each firm can anticipate the results that will take place, given its choices.

If there is a set of strategies with the property that no player can benefit from changing strategies mid-game, then that set of strategies and the corresponding payoffs constitute a Nash equilibrium.[9] Thus, the resultant Nash equilibria from this analysis are perfect in subgames. Each cell presents the profits of Firms 1 and 2, respectively, associated with each of their strategy choices.

The payoff matrix in Table 3-2 reveals that the dominant strategy for Firm 2 is to refuse the partnership exactly when Firm 1 considers the project without any type of cost for itself. The strategy for Firm 1 depends on the cost of the project in relation to the total cost of the tax: The bigger the differences between the emissions reduction technologies for the firms, the smaller the tax. A reduction in d, in turn, reduces k. Conversely, the bigger the differences between k, a_A, and a_B, the bigger the charged tax d. Still, no matter how much Firm 1 earns with the implementation of the project, the Nash equilibrium is achieved exactly when Firm 1 pays the environmental tax and Firm 2 benefits from the smaller market share taken by Firm 1, raising its profits in relation to the original equilibrium.

Therefore, a clear loss of well-being occurs in Country A as a result of the reduction in the profits of Firm 1. In Country B, there is a loss due to the price increase. As Firm 2's profits increase, it is necessary to verify which of the effects predominates. Since d is less than 1, its dimension relative to consumption a_i has to be significantly reduced, indicating that the ensuing social costs of demand reduction and price increase also have to be reduced. Only Firm 2's shareholders benefit from this result. Moreover, the increase in profit by Firm 2 is inferior to the profit loss of Firm 1; hence, the imposition of environmental policies in one of the countries causes a global loss of social welfare. These results indicate that a restrictive environmental policy in the developed country, in addition to a mechanism such as the CDM, instead of inducing a cost-efficient solution, would lead to a solution where there is loss of well-being.

The possibility that Firm 1 generates emissions reduction savings in a less technologically advanced country adds value to the excess emissions of Firm 2; Firm 2 is the one that is on the receiving end of the projects. In the model used here, this value is given by the extra profit that Firm 2 gets by inducing Firm 1 to pay an environmental tax. Firm 2 only accepts the project if Firm 1 offers a greater value than this extra profit for the right to implement a project of emissions reduction. In this case, it can be said that Firm 2 sells its excess of emissions to Firm 1.

Incentive Strategies' Effects

We now consider strategies that induce Firm 2 to cooperate when requested. As seen in Section 3, Firm 2 does not have any incentive to cooperate and allow the implementation of a project of emissions reduction. The imposition of an environmental tax on Firm 1 reduces its participation in the market, and part of this loss is reverted as profit to Firm 2. Allowing the project to be carried through, however, does not increase its profit.

For Firm 1, however, the choice between the project and the tax depends on the costs of each one. Inequality 4-1 indicates that Firm 1 will always prefer the project that has the lesser impact on its profit:

(4-1)

Inequality 4-1 does not have any effect on the result of the game; therefore, it is Firm 2's choice that determines the resultant equilibrium. Note that the application of Inequality 4-1 determines the resultant pair

of strategies, regardless of the fact that the payoffs associated with both of the two strategies are the same. In order to incorporate an incentive restriction to the problem, assume that Firm 2 agrees to allow the implementation of a project, as long as the tax loss of its extra profits is compensated. Thus, Firm 1 could add the extra profit of Firm 2 to the costs of the project k when it pays the environmental tax. Firm 1 would consider the viability of the project depending on the tax payment according to Inequality 4-2 or, alternatively, Inequality 4-2a.

(4-2)

(4-2a)

The idea is that Firm 2 accepts the project when Inequality 4-2a is true. The mechanism of incentive shown above completely modifies the resultant equilibria. The dominant strategy for Firm 2 is now to allow the implementation, and Inequality 4-2a determines game payoff. Assume that, for Firm 2, the implementation of the project without the loss of extra profits is preferable to the extra profits alone, either because of local emissions reduction or for a better image of the firm in the market. In the case that Firm 1 does not request the project, payoff for Firm 2 is indifferent.

When the inequality given by 4-2 or 4-2a is true, Firm 1 proposes the project to Firm 2. For such a proposal to carry through, the differences between project cost and the components dimension of stand-alone consumption for both markets a_i, in addition to the value of the environmental tax to be charged d, are determining.

For simplicity, denote the left side of Inequality 4-2a as $f(d, k, a_A, a_B, c)$, or $\hat{f}$ in short. Whenever $\hat{f}$ is less than 0, Firm 1 considers the project. An important point becomes visible from the graph of $\hat{f}$ when only the environmental tax is fixed, that is $(d_1 < d_2 < d_3 < d_n)$, as seen in Figure 4-1. The smaller the tax charged for particular values of k, the larger the probability that Firm 1 prefers this mechanism.

In Figure 4-1 the parameters that determine the shift to the right are the smaller size of the components of stand-alone consumption, a_A and a_B ,and the higher costs for the implementation of the project. Thus, once the environmental tax is fixed, Firm 1 observes the associated $f(d)$. It then decides whether to propose an environmental project to Firm 2 or whether it should pay the tax to Country A. The resultant equilibrium is then known. For example, if the cost of the project is very small relative to the components of stand-alone consumption, then the curve is similar to that of d_1, where even with lower taxes, the environmental project is the more viable alternative for Firm 1.

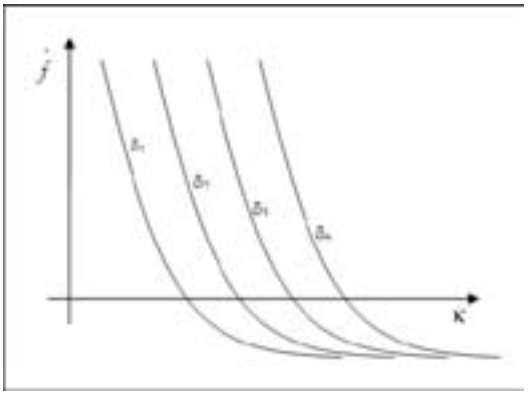

Figure 4-1. Effects of deferment taxes on project implementation likelihood.

An alternative use of Inequality 4-2 is to trigger the accomplishment of a project of environmental control in Country B or to trigger the reduction of local GGEs in Country A. Note that with payment of the tax, the amounts produced by Firm 1 are reduced and, consequently, its emissions decrease as well. Because all information is common knowledge, the agency has knowledge of Inequality 4-2 and would establish a tax in compliance with its objectives. Therefore, let d be such that $\hat{f} = 0$, as variations of this tax immediately affect the result of the game. In the specific case treated here, this tax is given by

(4-3)

Another result of using an incentive mechanism, such as that indicated by Inequality 4-2, is that the resultant final equilibrium can by and large be determined in the beginning of the game. Equation 4-4 is comparable to Equations 3-3, 3-3a, and 3-3b. The predicted profits for each firm associated with the equilibrium strategies

can then be expressed by Equations 4-4 and 4-5.

(4-4)

(4-5)

An Alternative Conjecture for Future Work

The final report of the Third Conference of Parts (COP3), carried through in Kyoto in 1997, considers four basic mechanisms for the global control of GGEs.[4] The approach intended in these mechanisms is guided toward the use of environmental markets, where the main contributory element of cost efficiency of this global policy is the ITQ market.

In order to finance the implementation of environmental control programs in developing countries, the CDM was included, by which the decurrent credits of reductions effected from designs implemented in these countries can be used by developed countries to their own advantage. Initially, this mechanism may be the one used because of the great cost savings it can generate, because environmental policies in developing countries are, in general, less rigid. It is also probable that these designs will be directly financed by developed countries or by firms located in these countries.

However, as Hahn and Stavins point out, the role of domestic politics is decisive for the success of the program, as a good part of the GGEs come from industrial processes. As discussed in the previous section, the imposition of one specific environmental policy in a developed country can generate, through the CDM, value for the excess of emissions in developing countries. The responsibility for the fulfillment of quotas agreed upon in Kyoto is transferred to the firms headquartered in developed countries; these can negotiate the right to carry through GGE reductions in developing countries.

Under particular hypotheses, the market is able to provide the necessary instruments to avoid compromising the efficiency derived from the propositions put forth in the Kyoto Protocol. This model, as presented in this paper, is only possible given the assumption regarding the nature of the information between the countries involved. Such assumptions indicate the fundamental role of local environmental authorities in propitiating conditions so that market crashes do not compromise the effectiveness of the program.

Hence, despite the fact that CDM presents a series of advantages for developing countries, some aspects of the partakers' behavior must be considered in relation to the practiced environmental policies in their countries. Individual actions by firms cannot harm the well-being of the population. Alternatives are the immediate mapping of the sources of GGEs in these countries and the imposition of referring external costs provoked by this type of pollution, in order to induce local firms to accept the implementation of designs of emissions control, financed by external resources.

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Appendix

Table A-1. A list of Annex-I countries, together with their relative greenhouse-gas emissions levels in 1990, taken from endnote 4.

Country	Emissions (Gg)	Percentage of Total Emissions
Australia	288,965	2.1
Austria	59,200	0.4
Belgium	113,405	0.8
Bulgaria	82,990	0.6
Canada	457,441	3.3
CzechRepublic	169,514	1.2
Denmark	52,100	0.4
Estonia	37,797	0.3
Finland	53,900	0.4
France	366,536	2.7
Germany	1,012,443	7.4
Greece	82,100	0.6
Hungary	71,673	0.5
Iceland	2,172	0.0
Ireland	30,719	0.2
Italy	428,941	3.1
Japan	1,173,360	8.5
Latvia	22,976	0.2
Liechtenstein	208	0.0
Luxemburg	11,343	0.1
Monaco	71	0.0
Netherlands	167,600	1.2
Norway	35,533	0.3
New Zealand	25,530	0.2
Poland	414,930	3.0
Portugal	42,148	0.3
Romania	171,103	1.2
Russia	2,388,720	17.4
Slovakia	58,278	0.4
Spain	260,654	1.9
Sweden	61,256	0.4
Switzerland	43,600	0.3
United Kingdom	584,078	4.3
United States of America	4,957,022	36.1
Total	13,728,306	100.0

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