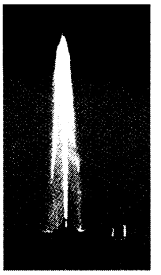
for 15 minutes and samples taken again to determine stress-induced corticosterone concentrations from handling.
4. L. M. Romero and M. Wikelski, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
5. General linear model repeated measures analysis of variance, $F(2,40) = 34.6$, $P < 0.001$; for oil versus non-oil, $P < 0.001$; for visible oil versus no visible oil, $P = 0.17$. Corticosterone levels were determined with a standard radioimmunoassay (assayed in quadruplicate). All samples were analyzed within one assay; intra-assay variation was 6 ± 0.3% SD, and the detection limit was 0.5 ng/ml.
6. The research described here was supported by the National Science Foundation under grant IBN-0118069.

Removing CO_2 from Lake Nyos in Cameroon

THE DEGASSING EXPERIMENT ONGOING AT Lake Nyos, Cameroon, is not "unprecedented," as John Pickrell says in his News of the Week article "Scientists begin taming killer lake" (9 Feb., p. 965). It was proved feasible, with pipes of the same diameter, at Monoun in 1992 and at Nyos in 1995. Water from the lake bottom travels up through the pipes, and the CO_2 is released in a jet of spray 45 meters high as it comes out of solution near the surface. Scaling up this experiment by adding five similar pipes will allow the elimination of most of the 300 million cubic meters of CO_2 currently dissolved in the lake's deep waters within 5 years.

There are concerns, however, that water discharged from the jet might play the role of cold rainwater, sometimes put forward as the ultimate cause of the Nyos and Monoun limnic eruption disasters. Indeed, the ~2500 square meters of the lake surface sprinkled by the jet receive about the equivalent of a heavy tropical rain (~8 centimeters per hour). But the water resulting from the removal of CO_2 is not "dense" water—it is lighter than the bottom water precisely because it is not laden with CO_2. Moreover, it has been found that expansion of the gas en route to the surface through the degassing pipe is almost isothermal because of the low gas/water mass ratio of the fluid being vented—the 1.8°C temperature drop of the two-phase flow along the pipe brings the 25.2°C bottom water to a temperature very close to the mean temperature of surface water (22° to 23°C). Also, because the jet is 0.1 in relative density, it breaks down as a hazy spray that thermally equilibrates swiftly with air and mixes uniformly with surface water. Even in a worst case scenario, the penetration depth of discharged water has been calculated to be above the chemocline currently existing at 44 meters (*1*). Therefore,



concerns about cool water sinking and disturbing deep layers as a consequence of the degassing process are not relevant.

MICHEL HALBWACHS,[1] JEAN-CHRISTOPHE SABROUX[2]

[1]Universite de Savoie, BP 1104 Savoie Technolac, F-73376 Le Bourget du Lac, France; e-mail: mhalb@mail.univ-savoie.fr. [2]Institut de Protection et de Sûreté Nucléaire, 389 Centre de Saclay, F-91191 Gif sur Yvette, France; e-mail: sabroux@accra.ipsn.fr

References and Notes
1. M. Kusakabe *et al.*, *J. Volcanol. Geotherm. Res.* **97**, 241 (2000).

Mutation in Embryonic Stem Cells

A SINGLE HUMAN EMBRYONIC STEM (ES) CELL might be modified in the laboratory to provide an unlimited supply of cells for therapy, according to Geron researcher Melissa Carpenter in the News Focus article "Stem cells: new excitement, persistent questions" (G. Vogel, 1 Dec., p. 1672). This seems, however, unlikely to be true. Mutations accumulate during DNA replication before cell division, at a rate of about 1 in 10 bil-

CREDIT: BERNARD CANNET/MAGMA PRODUCTION

lion base pairs (*1*). With 3 billion base pairs per cell in the human genome, about one in every three cell divisions results in a mutation. Cells derived from stem cells that have replicated through many generations will have accumulated mutations and be susceptible to cancer or have decreased viability. There is evidence that the body has evolved to discard a lineage from a particular stem cell for this reason, because replicative senescence has been shown to be controlled by the number of times a cell divides and not by its chronological age (*2*).

Expecting an unlimited supply of cells for therapy from a single ES cell is inconsistent with the nature of the evolution of stem cells. A tier organization of stem cells with sequentially diminishing potential is necessary for extended longevity in complex, multicellular organisms. Stem cells that supply cells for tissue therapy would need to be replaced periodically.

LOUIS ROCCANOVA,[1] PATRICIA RAMPHAL,[2] PHILIP RAPPA III[3]

[1]3988 Demont Road, Seaford, NY 11783, USA; e-mail: louisrocc@aol.com. [2]235 South 8th Street, Lindenhurst, NY 11757, USA; e-mail: pramphal_11757@yahoo.com. [3]5901 Mount Eagle Drive, Suite 1505, Alexandria, VA 22303, USA; e-mail: philip3@gateway.net

References and Notes

1. W. K. Purves *et al., Life: The Science of Biology* (Sinauer, Sunderland, MA, and Freeman, Salt Lake City, UT, 1998), p. 256.
2. J. Smith, O. M. Pereira-Smith, *Science* **273**, 63 (1996).

Response

EVIDENCE THAT THE EXPANSION OF HUMAN ES cells for therapeutic use will not be hampered by genetic mutation has been published in three sets of studies. First, late-passage murine ES (mES) cells (>250 doublings) and cloned mES cells after genetic manipulations extensively colonize embryos without signs of crisis or transformation (*1*). Second, early- and late-passage somatic cells, from fetal or old donors, can be used to create cloned animals by nuclear transfer at about equal efficiencies, and normal ES cells can be generated from clones (*2*). Third, telomerase-immortalized human cells are viable and nontransformed, and retain differentiated function, even after several hundred divisions (*3*). Human ES cells are telomerase positive, immortal, clonable, pluripotent cells that form mature tissues containing cells derived from all three embryonic germ layers when injected into immune-compromised mice, even after 250 doublings in culture (*4*).

For human therapeutic applications, Good Clinical Practice regulations will require the qualification of master and working cell banks. The limits for normal expansion of human ES cells have not yet been determined. To date, these cells are karyotypically normal after 250 population doublings in vitro (*4*). Within 150 doublings, there is ample room for genetic manipulation, clonal selection, quality testing, and creation of master and working cell banks for subsequent differentiation under standardized procedures. Even if 80 doublings were used in genetic manipulations, the last 70 doublings from a single cell would generate 10^{21} cells. And at 1% efficiency of the final differentiation process, this would yield enough cells to treat 10 million patients with 10^{12} cells (~1 kilogram each).

The real issue at hand is the growing, unmet clinical needs in chronic degenerative diseases that are inadequately addressed by conventional therapies. Organ dysfunction commonly results from cell death due to aging or chronic damage in tissues with inherently limited regenerative capacity (e.g., heart, brain, pancreas, kidney, and articular cartilage). The best available candidates for a source of safe, effective, and manufacturable replacement cells are embryonic and perhaps some somatic stem cells. Most somatic stem cells, however, are rare, appear to be telom-

erase negative, senesce within about 50 divisions, and are usually difficult to isolate and grow. Hematopoietic and mesenchymal stem cells have had limited success, in part because of their inability to be easily cloned, manipulated, and expanded. These are important features that limit the usefulness of somatic stem cells for the development of safe, efficacious, and cost-effective cell therapies for the millions of patients with chronic degenerative diseases.

In less than 3 years of work in a few laboratories, undifferentiated human ES cells have been shown to be genetically stable, immortal, clonable, and pluripotent (*4*). Clearly, not all questions are answered, but the point raised by Roccanova and coauthors will likely not significantly affect the use of human ES cells for human therapeutic applications.

CALVIN B. HARLEY, JANE S. LEBKOWSKI, MELISSA K. CARPENTER, THOMAS B. OKARMA

Geron Corporation, Menlo Park, CA, 94025, USA

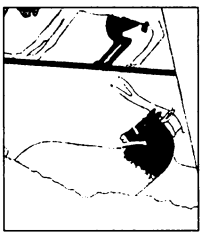
References and Notes

1. Y. Suda, M. Suzuki, Y. Ikawa, S. Aizawa, *J. Cell. Physiol.* **133**, 197 (1987); E. Wolf, R. Kramer, I. Polejaeva, H. Thoenen, G. Brem, *Transgen. Res.* **3**, 152 (1994).
2. M. J. Munsie *et al., Curr. Biol.* 10, 989 (2000); T. Wakayama, I. Rodriguez, A. C. F. Perry, R. Yanagimachi, P. Mombaerts, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 14984 (1999); W. M. Rideout III *et al., Nature Genet.* **24**, 109 (2000); R. P. Lanza *et al., Science* **288**, 665 (2000); E. Kawase, Y. Yamazaki, T. Yagi, R. Yanagimachi, R. A. Pedersen, *Genesis* **28**, 156 (2000).
3. X.-R. Jiang *et al., Nature Genet.* **21**, 111 (1999); C. P. Morales *et al., Nature Genet.* **21**, 115 (1999); M. M. Ouellette *et al., J. Biol. Chem.* **275**, 10072 (2000).
4. M. Amit *et al., Devel. Biol.* **227**, 271 (2000).

More About Duikers in Ancient Egypt

IN HIS LETTER "WERE THERE DUIKERS IN ancient Egypt?" (2 Mar., p. 1701), Nicolas Manlius describes a figure in a mural from an ancient Egyptian tomb that resembles the Jentink species of duiker (*Cephalophus jentinki*). Duikers have not been listed among the fauna of ancient Egypt, but on the basis of several lines of evidence, Manlius suggests there might have been an isolated duiker population that persisted in ancient Egypt north of other duiker populations and that served as the source for the mural figure.

While preparing my dissertation *The Funerary Sacrifice of Animals During the Predynastic Period* (*1*), I came across the following information. Although most animal burials (not to mention interment of disarticulated bones from food offerings) of the predynastic period [~3200 to



3050 B.C. (*2*)] were rarely identified as to species, in a few instances attempts were made to do so. Brunton reported the skull and leg bone of a duiker as a food offering in a predynastic grave at Matmar (in the Badari region) dated to the Naqada III period (*3*).

DIANE VICTORIA FLORES

Giza Archives Project, Museum of Fine Arts, 465 Huntington Avenue, Boston, MA 02115, USA. E-mail: dflores@mfa.org

References and Notes

1. D. V. Flores, thesis, Univ. of Toronto (1999).
2. Dates for the cultural phases of the predynastic period are a matter of debate. The dates given here are from B. Adams, *Predynastic Egypt* (Shire Publications, Buckinghamshire, U.K., 1988).
3. G. Brunton, *Matmar, British Museum Expeditions to Middle Egypt, 1929–1931* (Quaritch, London, 1948), p. 24 and p. 29. The grave described was an unregistered one in the 200 series.

CORRECTIONS AND CLARIFICATIONS

REPORTS: "X-ray pulses approaching the attosecond frontier" by M. Drescher *et al.* (9 Mar., p. 1923). In Figure 5, the number "76.0" at the base of the *y* axis should have read "75.0."

CREDIT: CLICHÉ BIBLIOTHÈQUE D'ART ET D'ARCHÉOLOGIE JACQUES DOUCET, PARIS