

Sweden has gone even further. It has ended a \$500,000 collaboration with India started last December in the fields of environment, energy, and food processing. And Mohan Gopal Kulkarni, a polymer chemist at the National Chemical Laboratory in Pune, says his collaborative project on biodegradable polymers with Y. Tokiwa of the National Institute of Biosciences & Human Technology in Tsukuba, Japan, has been delayed indefinitely "because of the recent nuclear tests."

Despite these setbacks, most Indian officials seem confident that their country can ride out the storm. Raghunath Anant Mashelkar, secretary of the department of scientific and industrial research, feels that "the new round of sanctions can easily be brushed aside, as India has literally grown up in this atmosphere of technology denials." But some are not so sure. They warn that Indian science will suffer as a result of this international isolation, and that the repercussions from the nuclear blast will be felt even if official sanctions are lifted soon. One engineer worries that civilian research will pay a heavy price for what he calls "the romantic indulgences of a few nuclear scientists."

—PALLAVA BAGLA

With reporting by Eliot Marshall.

ANIMAL CLONING

Cloned Mice Provide Company for Dolly

Dolly, the cloned sheep, can no longer be considered a fluke. As the first—and, at the time, only—mammal cloned from an adult cell, she was greeted first with awe and, later, with doubts (*Science*, 19 December 1997, p. 2038; 30 January, pp. 635 and 647). Dolly was up against the dogma that DNA from mature cells could not start over and guide an egg's development into a complex, multicellular organism. Before that dogma could be overturned, skeptics argued, the cloning experiment that yielded the lamb needed to be replicated. Now, they've gotten their wish.

In this week's issue of *Nature*, Ryuzo Yanagimachi and his team at the John A. Burns School of Medicine at the University of Hawaii, Honolulu, provide the first scientific report confirming that cloning from adult cells is not only possible but repeatable. In it, they describe experiments that have so far yielded more than 50 cloned mice. Two other reports in the same issue describe DNA analyses proving that Dolly and

the ewe she was cloned from are indeed genetically identical, as would be expected of clones. And in Japan, two calf clones born 5 July but not yet fully described in the scientific literature (*Science*, 10 July, p. 151) have apparently passed similar tests. "[Cloning] is a real phenomenon," comments Richard Schultz, a developmental biologist at the University of Pennsylvania, Philadelphia.

These achievements may reignite the ethical frenzy that followed the first reports of Dolly, primarily because of worries that the technology will be applied to humans. And they will certainly spur renewed vigor among companies vying to apply these technologies. For example, they might be used to clone herds of cattle that produce therapeutic proteins in their milk. "We intend to commercialize [the mouse technology] on a broad range of animals," says Laith Reynolds, CEO of ProBio America, a Honolulu-based company that is now supporting the mouse work in Hawaii.

To clone mice, Yanagimachi, working with Teruhiko Wakayama of the University of Tokyo in Japan, devised a variation of the technique used by Ian Wilmut of the Roslin Institute in Scotland and Keith Campbell of PPL Therapeutics to create Dolly. The idea is to get nuclei from adult cells into eggs whose own nuclei have been removed. The resulting cells can then be triggered to develop into embryos, which can be implanted in foster mothers. But while the Roslin team got the adult cells to fuse with enucleated eggs by subjecting them to an electrical pulse, Wakayama uses a very fine needle to take up the donor cell nucleus, which he very gently and quickly injects into an enucleated egg. "He is very careful to make sure as much of the donor cytoplasm is gone as possible," says Schultz. That cytoplasm could contain factors that might thwart proper development.

The Hawaii group also took a different approach to initiating egg development. In the Roslin team's case, the same electrical pulse that fused the cells prompted the egg's activation. Wakayama first lets the cells sit for up to 6 hours to give the egg cell time to alter the donated DNA so that its developmental genes can be expressed again. Then, the Honolulu team triggers development of the eggs by putting them into a culture medium containing strontium, which stimulates

the release of calcium from the eggs' internal stores—the same signal that tells fertilized eggs it is time to start dividing.

For some reason, the Honolulu team's strategy worked best with cumulus cells, which surround an egg as it matures. Over the past year, the group has used them to create some 50 clones, confirming their clonal origins by comparing the DNA of the newborn mice to that of the animals that provided the nuclei. The cloned mice seem normal: The group has cloned some clones and mated others, creating healthy young in both cases. All told, "it's a very compelling paper," says



Clones' clan. Two cloned mice stand by their white surrogate mother (lower tier), with the egg and nucleus donors above (top right and left, respectively).

Michael McClure, a cell biologist at the National Institute of Child Health and Human Development in Bethesda, Maryland.

Just as compelling are the results of two DNA analyses, conducted independently by the PPL-Roslin team and a group from the Hannah Research Institute in Ayr, Scotland, and the University of Leicester in the United Kingdom, to evaluate Dolly's origins. Some researchers thought that the limited DNA analysis Wilmut's team originally performed to show that Dolly is not an offspring of its surrogate mother was not convincing. So, the two groups made a more detailed comparison of DNAs from Dolly, from the cultured udder cells used as nuclear donors, and from the ewe that provided the cells.

Wilmut's team, working with a local company called Rosgen, analyzed 10 microsatellites—short stretches of DNA known to vary between unrelated individuals. "They all had identical patterns," comments Robert Wall, a geneticist at the U.S. Department of Agriculture in Beltsville, Maryland. The second team compared DNA fingerprints—patterns

created by chopping up DNA with enzymes and sorting the fragments by size—from the same three sources, and also looked at DNA obtained from animals belonging to the same herd as the ewe. Dolly's fingerprints matched the donor ewe's but not those of the herd. "These two [reports] are fairly powerful demonstrations that Dolly is what they say she is," Wall concludes.

The two Japanese calves, obtained by fusing oviduct cells from one cow with enucleated eggs from another, apparently are as well. Last week, Yukio Tsunoda, a professor of animal reproduction at Kinki University's Faculty of Agriculture in Nara, announced that DNA testing has confirmed that the calves are offspring of the oviduct cell donor. "I think there is no mistaking that they have repeated the Roslin procedure," says Tomohiro Kono, a developmental biologist at Tokyo University of Agriculture. The Japanese team, including researchers from both Kinki and the Ishikawa Prefectural Livestock Research Center in Nara, also said that they have an additional four cows pregnant with cloned embryos.

Now, cloning researchers can move on to other challenges, such as trying to improve their success rates, currently a few percent at most. This effort should be helped by the ability to study cloning in mice, which have shorter life cycles and require much less care and space than, say, sheep or cows. With improvements, says Wilmut, nuclear transfer "is going to be a very reliable, robust [cloning] method."

—ELIZABETH PENNISI

With reporting from Dennis Normile in Tokyo.

SPACE SCIENCE

Negative Review Galls Space Crystallographers

The promise of space-grown protein crystals has been a major selling point for the international space station. Larger and more perfect than Earth-grown versions, they could reveal new molecular details and new targets for drug designers. But a group of academic scientists who issued a review of the field last week said that crystal-growth experiments NASA has already flown aboard the space shuttle have not lived up to expectations. The seven members of the American Society for Cell Biology (ASCB) said in their report that the field has made "no serious contributions" to scien-

tific knowledge and there is "no justification" for continuing such studies in space.

Released on the eve of the House vote on NASA's 1999 budget, the report was distributed at a 15 July press conference at the Capitol by Representative Tim Roemer (D-IN) as he sought support for an amendment to eliminate the space station. At press time, the amendment was not expected to pass, but the report has infuriated some protein crystallographers. "I think the report is absolutely wrong," says Larry DeLucas, a crystallographer at the University of Alabama, Birmingham. "I can't believe the [ASCB] would get behind a statement like that." DeLucas says his NASA-funded space-based research helped reveal a protein structure that has contributed to the ongoing development of influenza drugs. And Daniel Carter, a biophysicist who formerly worked for NASA and now directs New Century Pharmaceuticals, a company in Huntsville, Alabama, that receives funding from NASA, says his space-based work crystallized proteins 10 times larger than those grown on the ground, making them available for structural studies. Of the ASCB report, Carter says, "it just seems to be more of an opinion than a review of the facts."

Members of ASCB's panel were unanimous in giving NASA's crystallography program bad marks. So far, the \$9-million-a-year effort has not lived up to claims that it would aid drug development for Alzheimer's disease and breast cancer, says the chair of ASCB's panel, biologist Donald Brown of the Carnegie Institution of Washington, D.C. "The [Earth-based] crystal community doesn't feel that real

gains have been made in space," says Brown. Another member of the panel, Harvard University crystallographer Stephen Harrison, says he conducted a literature search for crystals grown in microgravity conditions and determined that "none of the modest successes reported" had made a "significant impact" on drug design or structural biology.

A third member of the panel, Washington University biologist Ursula Goodenough, explains that "it became untenable for those of

ScienceScope

SOUNDING THE INFOTECH ALARM

The nation's information technology (IT) infrastructure is looking increasingly "fragile" in the face of the growing torrent of data it must process, a presidentially appointed panel warns in a forthcoming report. It recommends a doubling in federal R&D to more than \$2 billion a year.

According to a draft leaked to CNN, without more spending in areas such as high-end computing and software, the United States risks "being overcome by nations with a clearer plan and a stronger view of the future."

The big problem, says panel co-chair Ken Kennedy, head of the Rice University Center for Research on Parallel Computation in Houston, is that the size and rapidly increasing complexity of IT systems have "far outstripped the growth in research." Hence, key systems, such as those for air traffic control, are based on software that may be unreliable, insecure, error-prone, or difficult to upgrade. Kennedy also says the alleged shortage of IT workers in the U.S. is real and "very serious." The panel's interim report is due out early next month (see www.hpcc.gov).

COURT SIDES WITH TOBACCO

A court ruling has attacked a milestone Environmental Protection Agency (EPA) report that classified secondhand smoke as a human carcinogen. On 17 July, 5 years after the report came out, Judge William Os-teen of North Carolina's 4th U.S. District Court ruled in favor of tobacco company plaintiffs that the "EPA did not demonstrate a statistically significant association" between secondhand smoke and lung cancer. He also chastised the agency for being "publicly committed to a conclusion before research had begun."

The EPA study, one of several dozen to suggest a link between cancer and secondhand smoke, has been used to bolster lawsuits and is credited by some with the quick spread of indoor smoking bans nationwide. R. J. Reynolds now says it may challenge such ordinances.

EPA says it will appeal the ruling. But antismoking advocates such as Robert Kline, director of the Tobacco Control Legal Clinic at Northeastern University law school, contend it really doesn't matter. Says Kline, "It's going to be hard to put the genie back in the bottle."

Contributors: Constance Holden and Luis Campos



Disputed value. Lysozyme crystals 1 cm long grown aboard the Mir space station, and Representative Tim Roemer, who distributed negative report.