For decades, more and more researchers have been using fewer laboratory animals for compassion's sake. Thanks to new experimental techniques, many are getting cleaner results, too

Humane Science Finds Sharper and Kinder Tools

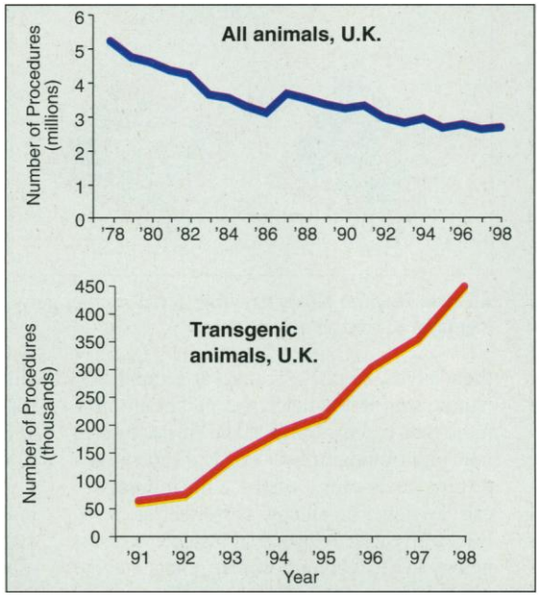
BOLOGNA, ITALY—A decade ago, veterinary surgeon Christian Schnell tested candidate drugs to lower blood pressure with a procedure that was highly stressful—for himself and his test animals. First, he would anesthetize marmosets and insert a catheter into an artery in their legs. The next day he restrained the conscious animals, orally administered the drug, and recorded blood pressure through the catheter for 4 to 5 hours. Not only did the harried animals' hearts race during the experiment, but each one could be used for only six trials before all its suitable arteries had been tapped.

But by 1991, Schnell, a researcher at the drug company Ciba-Geigy in Basel, Switzerland, switched to a new and more sophisticated technology: a sensor that he implanted in the animals' abdominal cavities. The device continually measures blood pressure and transmits the data to receivers in the cages, allowing the marmosets to move freely and remain with their families—more relaxed and with normal heart rates. Without the confounding effect of stress, the results are cleaner. "We are now convinced we're measuring the truth," says Schnell. And without the need for catheters, Schnell could do the same research with only 10% of the marmosets he had previously needed, saving the company up to $200,000 a year.

Schnell's case illustrates an accelerating trend in which new technology is helping researchers reduce their reliance on animal experiments, while at the same time improving their results. Although animal rights extremists continue to use violent and intimidatory tactics against researchers in many countries (see page 1059 and *Science*, 4 June, p. 1604), more moderate campaigners for animal welfare have for years been working with researchers to encourage this trend toward better experimental design and more humane techniques. The motto of this movement is "Humane science is better science," and its creed is the "three R's"—replacing laboratory animals, reducing their numbers, and refining techniques to minimize pain and suffering (*Science*, 11 October 1996, p. 168). The results have been striking: The use of lab animals has declined in many European countries—in some cases by as much as 50% over the past 2 decades.

As a result, the mood among the more than 800 researchers who gathered here recently for the Third World Congress on Alternatives and Animal Use in the Life Sciences was cautiously upbeat. They exchanged information on a variety of technologies—including implantable sensors like those Schnell uses and new imaging techniques to replace invasive procedures—that are already reducing the number of animals and lessening distress. And researchers reported progress in several areas—such as DNA arrays and tests using stem cells—that could help drug companies rule out dangerous compounds before they're tested in animals. "The spin-offs of molecular biology and biotechnology will have a great impact on [lowering] the use of lab animals," predicts geneticist Bert van Zutphen of Utrecht University in the Netherlands.

But not all the trends are downward. Many animal welfare researchers are alarmed by the imminent prospect of a new round of toxicity tests in the United States on a host of so-called high production volume chemicals (see sidebar), as well as tests on potential endocrine disrupters, that may require millions of laboratory animals. And in some hot areas of research, such as transgenics, animal experimentation is rising fast. Since 1990, the number of procedures on transgenic animals in the United Kingdom, for example, has risen almost 10-fold to more than 447,000. That's "a huge rise and due to get much higher," predicts Caren Broadhead of the Fund for the Replacement of Animals in Medical Experiments in Nottingham.



Rebound? After a long decline in overall animal use (above), booming transgenic research may boost overall numbers in the U.K. and elsewhere.

Even when researchers come up with technologies that can lessen the use and suffering of test animals, they still face a formidable obstacle: the glacial pace of regulatory bodies in accepting replacement tests, such as cell cultures. "A validation study takes a long time," says Herman Koëter, principal administrator of the Environmental Health and Safety Division of the Organization for Economic Cooperation and Development in Paris. "You need years and years to get a gold standard." That frustrates researchers. "If people knew how onerous it can be to get a test validated, many fewer would begin developing new ones," says Ian Kimber, research manager of AstraZeneca's Central Toxicology Laboratory in Alderley Park, U.K.

Less is more

Schnell's work with marmosets to test potential blood pressure drugs is Exhibit A in support of the humane science movement's claim that compassion can improve science. Ciba-Geigy had been puzzled by the fact that some candidate compounds that had looked promising in the earlier, more invasive, tests were duds in early human trials. But when Schnell tried those compounds again using implanted monitors in unrestrained marmosets, they proved to be 10 times less effective at lowering blood pressure than they had in the restrained animals. "It was a shock when we discovered this," recalls Schnell.

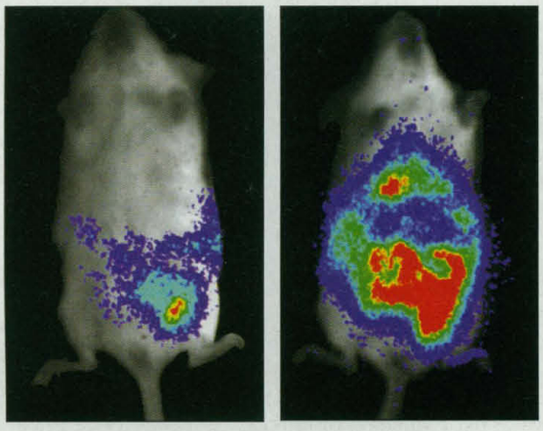
Since those early tests, telemetry sensors

CREDIT: U.K. HOME OFFICE

have shrunk in size and price and they are becoming more widespread. Blood pressure monitors weighing 3.5 grams are now small enough to be implanted into mice, and the device that Schnell uses costs about $3000. The new monitors are also far more versatile: Implantable devices can record temperature, blood pressure, heart rate, electrocardiograms, and intraocular pressure, and blood flow monitors will be available soon. "I'm convinced that telemetry will be the standard method in the near future," says Schnell.

Whereas implantable monitors can keep track of an animal's physiology, an imaging technique developed by Xenogen Corp. of Alameda, California, allows researchers to chart the course of an infection or the growth of tumors without any surgery at all. The technique essentially records a glow from inside the animal. The light bulb is the luciferase gene, which produces the firefly's bioluminescent protein. Researchers infect an animal with a microbe engineered to express luciferase, anesthetize it, and place it in a dark chamber. Some of the photons from the luciferase pass through the animal's flesh, and a charged-coupled device counts them for a few minutes, pinpointing the active microbes.

The pharmaceutical industry is eyeing this technology as a potential replacement for a standard test called the mouse thigh model. To check out new antibiotics, for example, technicians give the test drug to 14 or more infected mice, then kill a pair of the animals every 2 hours, grind up their thigh muscles, and culture microbes from the tissue over 2 days. The better the antibiotic, the fewer microbes grow on the ground-up muscle. In contrast, researchers can scan a living mouse in just 5 minutes. And measuring the same animal throughout the study—rather than comparing individuals that might have had slightly varying initial infections or responses to the drug—also reduces variability.



Glowing success. Fewer animals must die when researchers watch infections progress without dissection.

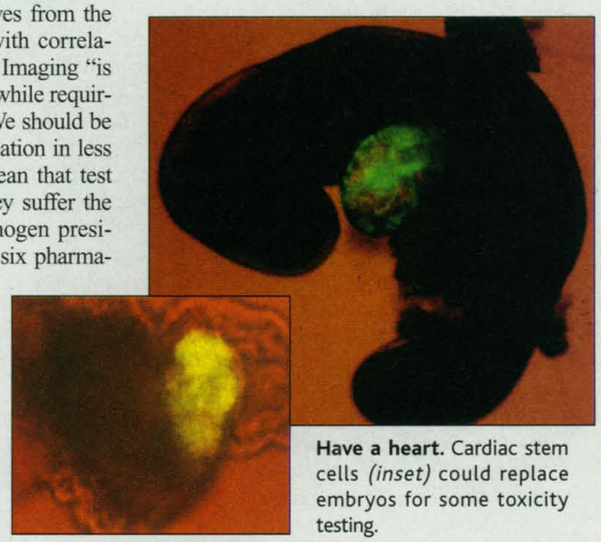
CREDITS: (LEFT TO RIGHT) C. BELLINGER-KAWAHARA, XENOGEN; (INSET) B. FLEISCHMANN, U. OF COLOGNE; M. FLEISCHMANN, U. OF VIENNA

One group of researchers, led by Tom Parr of Lilly Research Laboratories in Indianapolis, recently compared the two techniques and presented their results at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy in San Francisco in September. The team ran a mouse thigh model using doses of a known antibiotic, but before extracting the muscle, they imaged the animals. The dose-response curves from the two assays were very similar, with correlations ranging from 0.94 to 0.98. Imaging "is more sensitive and more precise while requiring fewer animals," says Parr. "We should be able to get more valuable information in less time." The quick results also mean that test animals can be killed before they suffer the full effects of an infection. Xenogen president Pamela Reilly Contag says six pharmaceutical companies, including Eli Lilly, are evaluating the technology, and 10 others are in various stages of negotiation.

Going in vitro

Drug companies are also showing interest in alternatives to animal tests to screen compounds for effects on fetal development. Researchers currently test for potential teratogenic effects by treating pregnant animals with a candidate drug and then checking embryos for abnormalities—a time-consuming and expensive proposition. "Most companies now want to have short tests that give a clear answer and that require small amounts of compound," says Philippe Vanparys, director of genetic and in vitro toxicology at Janssen Research Foundation in Beerse, Belgium. Recent developments in establishing immortal lines of stem cells—general-purpose embryonic cells that can develop into any type of cell in the body—have raised hopes that such tests may be feasible.

Because stem cells have a very reliable pattern of development into tissue, researchers can precisely measure any disruption to the number of cells, the quality of cells, and the timing of development. This provides a way of looking for subtle chemical effects that might lead to birth defects in particular organs. For example, Anna Wobus of the Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany, has developed an in vitro method to differentiate mouse embryonic stem cells into heart muscle cells, among others. Once these cells begin to beat after 9 days of normal development, researchers can check for defects in the nascent heart. In 1996, Horst Spielmann, director of the National Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments in Berlin, submitted this test to the European Centre for the Validation of Alternative Methods (ECVAM) in Ispra, Italy, an organization run by the European Union that assesses the suitability of in vitro tests for replacing established animal tests. "So far it looks very promising," says Juergen Hescheler, a molecular biologist at the University of Cologne, Germany.



Have a heart. Cardiac stem cells *(inset)* could replace embryos for some toxicity testing.

Now, Hescheler and his colleagues have added a feature to the test that could make it even faster, easier to use, and more versatile. At the Bologna meeting, he reported that his group has spliced a fluorescent reporter gene to the cardiac-specific promoter gene, so the cells express a green fluorescent protein on day 4 of development, cutting experimental time in half. "We can directly measure cell differentiation without any staining, so it's less time-consuming," says Hescheler. The team now wants to link reporter genes to other types of stem cells, such as neuronal, epithelial, and cartilage precursor cells. If the reporter proteins could fluoresce in different colors, scientists might be able to examine the effects of potential toxicants on a suite of tissues at once. Interest in the cardiac reporter is already high. "In the last month, I had five to six pharmaceutical companies asking for this test," says Susanne Bremer of ECVAM.

Toxic chips

Toxicologists are also turning to a hot new genetics technology to study cellular responses to test compounds: DNA microarrays, which are commonly used to track patterns of gene expression (*Science*, 15 October, p. 444). A single DNA "chip" carries an array of hundreds or thousands of short strands of DNA, each of which acts as a probe for a specific gene. To tell which genes were active

Toxicity Testing: The Many Arts of Persuasion

Last October, Vice President Al Gore announced what sounded like a great idea, one that won him plaudits from the environmental movement: a 6-year program to rapidly collect health and safety data on 2800 major industrial chemicals. But one lobby's triumph turned out to be another's catastrophe. Animal rights groups quickly denounced the High Production Volume (HPV) Challenge because they estimated it would require the destruction of more than a million animals. The groups charged that the killing of so many animals was needless because much of the information already existed, more could be derived from nonanimal tests, and some simply wasn't worth collecting.

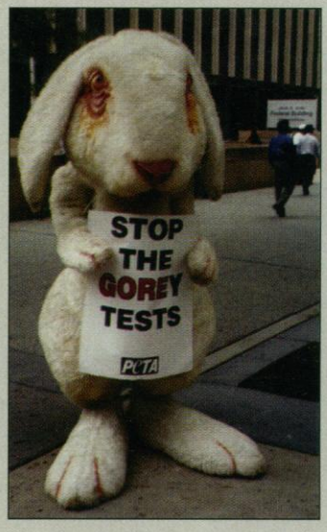
Over the past year, the lobbying to halt or modify the original plans of the HPV program has been intense. Gore has been followed around the country by a heckler in a rabbit suit, animal rights groups have taken out emotive newspaper ads, and animal welfare researchers have held workshops to offer Environmental Protection Agency (EPA) representatives technical advice on alternatives to animal testing. The combination may have worked. On 14 October, EPA sent a letter to 223 companies with new recommendations for testing HPV that should reduce animal use.

The impetus for the HPV program was a 1997 study called "Toxic Ignorance" by the Environmental Defense Fund, an advocacy group based in New York City. The report suggested that basic toxicology data were not publicly available for most of the chemicals that are manufactured or imported into the United States in amounts greater than 450,000 kilograms (1 million pounds) per year. In a quick follow-up review, EPA could only complete a checklist of specific health data for 7% of these chemicals. The agency then invited chemical manufacturers and importers to volunteer the basic toxicity data and test plans—or face regulation.

Activists countered that many tests were unnecessary, because some of these chemicals were either clearly safe, such as those already approved by the Food and Drug Administration for consumption, or obviously toxic, such as rat poison and turpentine. And for other compounds, they argued, EPA simply hadn't looked in the right databases. Many concerned scientists also weren't pleased that a fill-in-the-box suite of information was being required for all the chemicals. The HPV program "is bad news for those of us who seek a scientifically rational approach to hazard prediction and risk assessment," was the opinion of Michael Balls, director of the European Centre for the Validation of Alternative Methods.

In December, EPA held a stakeholder meeting, during which the agency and advocacy groups discussed the use of nonanimal tests in the HPV program. A month later, EPA representatives participated in a conference, called TestSmart, sponsored by the Center for Alternatives to Animal Testing (CAAT) at The Johns Hopkins University in Baltimore to brainstorm suitable alternative methods. Afterward, EPA tentatively proposed several approaches, such as combining reproductive and developmental toxicity tests or changing protocols (for example, replacing the infamous LD-50 test, which determines the dose at which half the treated mice die, with a test that requires fewer animals). Altogether, the agency said, this could reduce animal usage by up to 80%. People for the Ethical Treatment of Animals (PETA) disputed that estimate and kept up the pressure.

In its letter of 3 weeks ago, EPA officially responded to the concerns of the animal rights groups and made several recommendations to chemical companies. "We are trying to minimize the number of animals and avoid needless testing," explains Susan Wayland, the EPA deputy assistant administrator who signed the letter. "We just needed to write it down in a way that was clear." In accordance with international animal welfare guidelines, the letter discourages or rules out several animal tests, such as those for the reproductive effect of chemicals unlikely to be released from factories. To better ensure that no tests are redundant, the agency will now consider previous results from additional databases, including a widely used international chemical safety database called IUCLID.



Hopping mad. Some groups apply high-profile pressure.

EPA also postponed testing some chemicals for several years, in the hope that validated nonanimal tests for some may be available soon. "This is for us a compromise," says Mary Beth Sweetland, spokesperson for PETA, "but it's so much better than the slaughter that was going to take place." To help the search for alternatives, EPA announced that the National Institute of Environmental Health Sciences will invest at least $4.5 million during the next 2 years to develop and validate nonanimal protocols. EPA will chip in $250,000 and will seek to contribute about the same next year.

Although some activists still aren't completely satisfied with the outcome or the process—PETA claims the agency had to be dragged "kicking and screaming" to consider alternatives—the recommendations leave CAAT director Alan Goldberg feeling optimistic: "This is a major regulatory agency that has been taking a hard look at how to incorporate the best technology that is more humane." **–E.S.**

in a sample, researchers convert messenger RNA to complementary DNA, tag it with a fluorescent marker, and wash the sample over the chip. The cDNA sticks to a specific probe on the chip, and its presence is revealed by a glowing patch when the chip is illuminated with light.

Many toxicologists believe that such arrays could reveal which genes a cell turns on in response to toxic compounds—and because they directly probe the activity of human cells, the arrays may eventually be better than animal tests in predicting toxicity to humans. "DNA chips will be the source of the next reduction in animals used," predicts Spielmann.

AstraZeneca's Central Toxicology Laboratory (CTL) is one of the first off the blocks with a chip outfitted with DNA from 600 genes, associated with everything from cell adhesion and ion channels, to metabolism and immune response—all thought to be involved in cellular response to toxicity. "The most exciting thing about toxicogenomics is that we're going to start investigating genes we never would have thought of looking at," says CTL's Kimber. "That's where the big surprises—and big benefits—are going to come from."

Not everyone is convinced by the promise of DNA chips, however. "There's much hype about gene chip technology," says molecular biologist Johannes Doehmer of the Technical University of Munich in Germany. "They're very expensive, and it will take a few years before you can rely on them." And although the microarrays generate a lot of information very quickly, the results can be hard to interpret. "The vast majority of our time is [spent]

CREDIT: PETA

figuring out the gene response," says CTL's William Pennie.

Even though many researchers say that animals will never be replaced for conducting general investigations or checking a whole-body response to a potential toxicant, scientists are also enthusiastic about the potential of chip technology and in vitro tests for asking specific questions—with data from human cells, rather than animal models of disease. "We can now go into more depth," says toxicologist Sandra Coecke of ECVAM. "With in vivo tests, you ended up with kind of a black box." Indeed, Coecke and others feel that these kinds of new methods—once validated—could not only replace animals tests, they could be an improvement. –ERIK STOKSTAD

MEETING SOCIETY OF VERTEBRATE PALEONTOLOGY

The Stories Behind The Bones

DENVER, COLORADO—There's more to paleontology than fossils, as was shown here on 20 to 23 October at the 59th meeting of the Society of Vertebrate Paleontology (SVP). Genetics labs, for example, uncovered an Ice Age disease; a changing atmosphere was fingered as the force behind the evolution of mammals in North America; and dissecting modern animals has hinted at the reason dinosaurs had such big noses.

Ancient Tuberculosis Identified?

The world's deadliest infectious disease, tuberculosis plagues a third of all people on Earth, killing 3 million every year. Exactly how the scourge first got a toehold in our species has been a mystery, but at the meeting researchers made a controversial announcement that they had a clue—in the form of DNA from *Mycobacterium tuberculosis* dating back 17,000 years.

For decades, the traditional story of TB had it arising in Old World pastures. Cows and their bovine relatives carry strains of the mycobacterium that are closely related to the human form; people could have become new hosts for TB when they began herding cattle and handling meat and hides. That idea seemed to find support in the devastating epidemics that swept through Native American society when European colonists arrived in the New World. Native Americans, never having domesticated cattle, had apparently been spared the disease until then and thus had immune systems that couldn't cope with TB.

But in 1994, that notion collapsed with the discovery of *M. tuberculosis* in a 1000-year-old mummy in Peru—predating Columbus's arrival by 500 years (*Science*, 25 March 1994, p. 1686). Native Americans carried TB long before Europeans came on the scene, and the massive epidemics that followed the contact could have resulted from overcrowding, malnutrition, and bad sanitation.

So where did the New World TB come from? In the late 1980s Larry Martin, a paleontologist at the University of Kansas, Lawrence, and Bruce Rothschild, an expert on ancient diseases at the Northeastern Ohio Universities College of Medicine in Rootstown, looked for evidence of TB on bones from the New World's grazing animals. The disease can leave scars on bones in places where the immune system has walled off infected cells. Martin and Rothschild examined bones of bison, musk ox, and bighorn sheep from the Natural Trap Cave in Wyoming, dating back 15,000 to 20,000 years. They found the scars in abundance but couldn't say whether the animals suffered from TB or other lesion-forming diseases, such as brucellosis. Because the lesions looked more like those of TB than of other diseases, "we knew it was a likely diagnosis," says Martin. "But we knew we could be wrong."

CREDIT: B. ROTHSCHILD

Old scourge. TB appears to be to blame for these lesions on a 17,000-year-old bison bone.

The researchers suspected that they might be able to settle the issue by finding genetic material from the mycobacterium itself in the fossils. Natural Trap Cave gets its name from the way it is entered—by falling into the entrance and dropping 30 meters to the cave floor. Animals have been falling to their death in the cave for 100,000 years. During the Ice Age, these unlucky creatures would have crashed into a heap of snow on the cave floor and been freeze-dried. There was a chance, therefore, that some of their DNA could have survived until now.

Rothschild and Martin extracted some of the bone tissue from a lesion on a 17,000-year-old bison. They sent samples to labs in Israel and England, each of which used the polymerase chain reaction to amplify any fragments of genes. As Martin explained in his talk, both teams identified genes belonging to *Mycobacterium*. Although the timing of human arrival in the Western Hemisphere is still under intense debate, Martin says, "my suspicion is that tuberculosis was waiting for humans when they came."

Based on the talk, however, other researchers are skeptical. "They didn't put all their ducks in a row," contends Ross MacPhee of the American Museum of Natural History in New York City. Many species of mycobacteria live in the soil, he points out, and they might have gotten into the cave and contaminated the bison material. Contamination has proven to be a big headache for scientists who study ancient DNA, yet Martin and Rothschild didn't present any control tests that could have ruled it out—for example, testing the bones of animals that don't get TB for the presence of the mycobacterium. "The result is really interesting, so why didn't they go that extra step and knock out the ambiguity?" asks MacPhee. According to Martin, his team will soon present data that address this issue.

If Martin and Rothschild are right, New World TB must have come from the Old World, when some infected mammals crossed the Bering Land Bridge and then infected the early Americans who hunted them. And if people in the New World picked up the disease from hunting, rather than farming, maybe the same goes for the Old World, too. The two researchers note that their scenario resembles current theories that trace AIDS in humans to hunting chimps and monkeys. "It shows that even for the most sophisticated side of medicine, it's useful to know what happened 17,000 years ago," says Rothschild.

Where Have All the Browsers Gone?

Two artist's conceptions, common in Earth history texts, tell the story. One portrays North America 20 million years ago in the early Miocene, showing a mix of grassland and trees, with many sorts of hoofed mammals, or ungulates, craning their necks to browse on leaves. In the second,