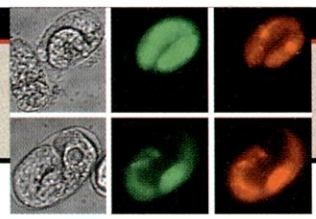
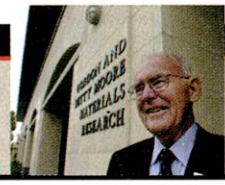


PAGE 1478
Color 'round the clock



1481
Moore for science

LABORATORY ANIMALS

Researchers Pained by Effort to Define Distress Precisely

When does stress turn into distress? The U.S. government wants to clarify that question and others affecting the care of millions of animals being used in research. But the 2600 pieces of advice it received during a 4-month period that ended earlier this month suggest its job won't be easy. The comments highlight a deep split between animal activists, who see the potential new regulations as a step toward eliminating all painful procedures, and most researchers, who say that the present system is working well and that no major changes are needed.

The U.S. Department of Agriculture (USDA) requested the comments to help it decide whether to adopt a formal definition of "distress" as part of its responsibilities under the Animal Welfare Act. Although the act seeks to "minimize pain and distress," says Ron DeHaven, deputy administrator of USDA's Animal and Plant Health Inspection Service (APHIS), the regulations to date have "focused on pain and have not given equal consideration to distress." To remedy that situation, APHIS came up with a working definition of distress: "a state in which an animal cannot escape from or adapt to the internal or external stressors or conditions it experiences, resulting in negative effects on its well-being." In July it asked for public comments, the first step in a long process that will likely culminate in new regulations.

APHIS would also like to fill in what DeHaven calls "gaps" in the reporting system used by research facilities. Current regulations require the labs to divide animals into three categories: those that do not undergo painful procedures; those that do and are given pain-relieving drugs; and those used in experiments, such as rodents for drug testing, that preclude relief because it would compromise the study. APHIS says these categories fall short by ignoring the duration and intensity of pain, the effectiveness of pain control, and palliative measures other than painkillers, anesthetics, or tranquilizers.

The comment period ended on 7 November. Although the volume paled in comparison with the torrent of reaction, most of it negative, to the government's proposal to add rats, mice, and birds to the welfare act (*Science*, 6 October, p. 23), some researchers believe that the changes could have an even greater impact on science.

Pained expression. Porphyrin staining around a rat's eyes could be part of a new definition of distress for lab rats.

They cover "not just reporting activities but also ... how the IACUCs [institutional animal care and use committees] do their business," says immunologist Robert Rich of Emory University in Atlanta, Georgia, president-elect of the Federation of American Societies for Experimental Biology (FASEB). Detailed regulatory prescriptions, he and others argue, might make the paperwork load intolerable and hobble IACUCs in exercising their best scientific judgment.

Researchers also question APHIS's attempt to define distress. It is "vague and could lead to widely varying, highly subjective interpretations," FASEB wrote USDA. "There are no simple physiological or behavioral criteria to mark the point where an animal that experiences stress becomes distressed."

Scientists are less united on how best to classify animals' exposure to pain and distress. "A lot of what is being talked about is already in place in the labs of responsible investigators," says Randall Nelson of the University of Tennessee, Memphis, chair of the council for the Institute for Laboratory Animal Resources at the National Research Council. In addition to arguing that further tinkering with the rules is unnecessary, many researchers accuse APHIS of bowing to pressure from the animal rights community. It's "all part of a plan to stop all biomedical research" by drowning it in costly bureaucracy, says Joseph R. Haywood, a pharmacology professor at the University of Texas Health Science Center in San Antonio.

Animal welfare groups don't deny that a discussion about new rules provides an opportunity to advance their agendas, which range from reduction to outright elimination of the use of animals in research. The Humane Society of the United States (HSUS), for example, wants to eradicate animal pain and distress by 2020 as part of a larger effort to "eliminate harm to animals" in research, education, and testing. Toward that goal, the society strongly favors more explicit rules for pain and distress reporting. HSUS has proposed a system, similar to one in the United Kingdom, that would group animals that undergo procedures without painkilling drugs into three classes based on whether the pain is mild, moderate, or severe.

Some scientists also support refining the reporting categories. Anesthesiologist Alicia Karas of Tufts School of Veterinary Medicine in North Grafton, Massachusetts, for example, says that changes would help both APHIS inspectors and lab workers by making the monitoring system more objective and systematic. Typically, says Karas, "somebody walks by and makes a check mark somewhere to see if an animal looks OK after a procedure." She has developed a checklist for research dogs at Tufts that covers physical problems, such as diarrhea and loss of appetite, and behavioral signs, such as irritability.

Other scientists have been working on objective indices of rodent pain. David Morton of the University of Birmingham in the U.K., for example, has developed a form for rats that covers behavioral signs such as not grooming, "walking on tiptoe," squeaking on being touched, and observations of "hunched posture," scruffy coat, and weight loss. He believes that forms adapted for a number of species including fish and pigs "have improved animal care, because they indicate in an objective fashion when animals are not 'right.'"

DeHaven predicts that the "smoldering" issue of pain and distress will become

CREDIT: PAUL FLECKNELL

increasingly visible in the next few years. It may also become even more contentious. With research in the postgenome world demanding a skyrocketing number of rats and mice, says Rich, "anyone who believes that we're going to eliminate pain and distress over the next 20 years just does not understand biological science."

–CONSTANCE HOLDEN

MICROBIOLOGY

Unlocking the Secrets Of the Grim Reaper

During the 14th century, the Black Death swept Asia and Europe, littering streets with corpses. Why, the people wondered? Was the Plague the work of an angry God? A medieval curse? As it happens, the real culprit was a tiny bacterium: *Yersinia pestis*. Centuries later, scientists are still learning how *Y. pestis* does its dirty work. Now, on page 1594, a new study adds another piece to the puzzle. The work may also shed light on the modus operandi of other noxious bacteria.

In the study, researchers led by biochemist Jack Dixon of the University of Michigan, Ann Arbor, unveil the workings of YopJ, one of a group of nefarious proteins that *Yersinia* bacteria, including *Y. pestis*, deploy. Early in the disease, *Yersinia* bacteria inject YopJ into macrophage cells of the immune system, beginning the cycle of destruction. There, the researchers have now found, YopJ acts like scissors—or more precisely, a cysteine protease—cleaving the macrophage proteins that would normally send an SOS to other immune cells for help. "YopJ cuts the macrophage's communication lines," says Dixon. Leaving the macrophage stranded, he adds, YopJ clears the way for other *Yersinia* proteins to destroy the cell.

"Very nice," says microbiologist Brett Finlay, of the University of British Columbia in Vancouver, about the new study. Bit by bit, Finlay says, researchers are unraveling the half-dozen virulent proteins that pack *Yersinia*'s punch. "This work takes us a step farther, defining the molecular machinery that allows YopJ to quiet the immune system," Finlay says.

Yersinia is infamous for causing the bubonic plague, which still affects at least 1000 people a year—and its brilliant biochemistry. Rather than climb inside macrophage cells, the bacteria hover outside and, like a molecular syringe, shoot six Yops (*Yersinia* outer proteins) into the cells. In itself a team of assassins, each Yop protein plays a distinct role in killing the cell. Last year, Dixon and his colleagues reported (*Science*, 17 September 1999, p. 1920) that YopJ blocks two signaling pathways—mitogen-activated protein kinase (MAPK) and nuclear factor κB—that the macrophage activates to send an SOS, in the form of tumor necrosis factor (TNF), to the immune system's B and T cells.

But how, exactly, does YopJ accomplish this? That question inspired the new study. To find out how YopJ works, the researchers ran computer programs that predicted YopJ's structure, based on its amino acid sequence. That analysis showed that YopJ closely resembles adenovirus protease (AVP), a well-known viral protein, as well as the AvrBsT protein in plant bacteria. These proteins all share a stretch of four amino acids—and it's this conserved catalytic region, Dixon knew, that makes AVP a cysteine protease and determines which substrates it can cleave. By extension, he reasoned, YopJ and AvrBsT must also be cysteine proteases, acting on similar substrates.

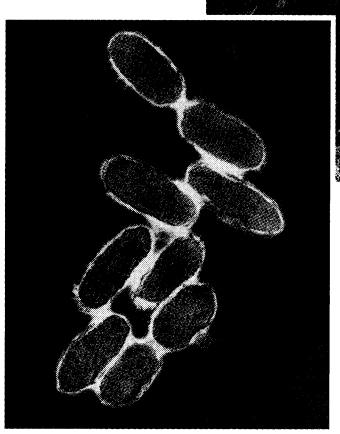
To test that idea, the researchers created three YopJ mutants, each altered in the key catalytic region shared with AVP. Without this region intact, the team found, each YopJ mutant failed to block the MAPK pathway—and stop production of TNF—in macrophage cells. By contrast, wild-type YopJ proteins stopped MAPK activity cold, silencing the macrophage's call for help. "Without this working catalytic site," Dixon concludes, "YopJ can't do its job."

To hammer the point home, Dixon decided to test the same mutations in AvrBsT, the plant homolog. He called Brian Staskawicz, a plant biologist at the University of California, Berkeley. And sure enough, when Staskawicz's lab mutated the same catalytic region in AvrBsT, that protein also failed to provoke its usual host response—in this case, localized cell death in tobacco leaves. Like YopJ, AvrBsT appears to be a cysteine protease, Staskawicz says. The first lurks behind the Black Death; the second, black spot disease. What's striking, adds Staskawicz, is that bacteria have evolved to unleash the same biochemistry in such different settings. Dixon adds: "Basically, the same thing that killed millions in Europe is sitting in your front yard." Not to mention elsewhere—YopJ homologs can also be found in bacteria such as salmonella and *Rhizobium*.

The *Yersinia* saga continues. Dixon's team is still searching for the unlucky host proteins cleaved by YopJ and AvrBsT. The current study suggests that both clip an assortment of so-called ubiquitin-like proteins used for signaling inside cells. The researchers hope to catch this action in vitro. Until they do, some hesitate to label YopJ a cysteine protease. "This is a very interesting idea, but more work needs to be done," cautions microbiologist Olaf Schneewind of the University of California, Los Angeles. Even now, *Y. pestis* perplexes the crowd.

–KATHRYN BROWN

Kathryn Brown is a writer in Alexandria, Virginia.



Black Death. *Yersinia* bacteria (left) caused the bubonic plague that ravaged Europe in the 14th century. Italian artist Francesco Traini depicted the plague in this 14th century painting, "Triumph of Death."

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