Out of the cold

Warmer living conditions could make lab mice better models of human disease.

Esther Landhuis

When tumor immunologist Elizabeth Repasky chats with friends and colleagues getting treated for cancer, she hears a common complaint: "I'm fine except I always feel cold now."

That comment caught Repasky's attention because her lab at Roswell Park Comprehensive Cancer Center in Buffalo, New York, has spent years warming up lab mice. Her mouse experiments aim to unravel mechanisms behind hyperthermia therapy, a rare kind of cancer treatment that kills tumors by heating them. Thermal therapies generally focus heat at a certain spot, but Repasky is onto something different—raising the core body temperature a few degrees to see if that could boost immune defenses, particularly those that slow tumor growth.

In a 2013 mouse study that stunned the scientific community, the Roswell researchers showed their hunch was right. When they injected mice with tumor cells and housed the animals at 30–31 °C—balmy conditions compared to most facilities' norm (20–26 °C)—they mounted immune responses that slowed tumor growth[1].

But there was a twist: "If you measure their body temperature, it looks normal," Repasky says. "That's because they expend a lot of energy to stay warm." When allowed to roam in a thermal apparatus with chambers set to varying temperatures, tumor-bearing mice spent more time in warmer (30–31 °C) areas, compared to cancer-free littermates. To Repasky that behavior seemed consistent with the complaints she heard from patients. "Having cancer makes mice feel colder too," she says. And when energy goes toward keeping warm, she notes, not as much remains for other functions, such as tumor-fighting immune responses.

In other words, under standard lab conditions (20–26 °C) mice can be cold-stressed. And depending on what their mice are meant to model, scientists may need to consider whether—and how—to raise the thermostat.

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Thermoneutrality

Within a certain range of ambient temperatures, known as the thermoneutral zone, an animal can maintain body temperature without upping its metabolism. For mice that preferred range is 30–32 °C[2]. For their human handlers, who are fully clothed and often wearing lab gowns, gloves, and face masks, thermoneutral is closer to 22 °C. If rooms were kept at mouse-preferred temperatures, lab personnel who are "in there changing cages and working all day long… would be sweating profusely and not very happy about it," says Chris Gordon, a research physiologist at the US Environmental Protection Agency who published the 1993 book, *Temperature Regulation in Laboratory Rodents*.

Since humans control the thermostat, lab mice must adapt—and for decades scientists have known they can do so without obvious harm to their well-being. But that doesn't mean their underlying physiological and metabolic responses aren't affected.

Ajay Chawla, an immunologist at the University of California at San Francisco who studies how immune cells control metabolism and tissue regeneration, became interested in thermoregulation about a decade ago when he saw papers about its impact on animal physiology. "There were some reports suggesting it was important, but the immunology field had not fully embraced it," he says. "Nobody was really doing much about it. Yet it has a huge effect."

He remembers, in particular, a 2008 study with results so dramatic they made him rethink his experiments. That study showed that turning up the mouse housing temperature from 20 to 30 °C caused the animals' heart rate to drop from 550–600 to 350 beats per minute. And conversely, if the housing temperature was decreased to 20 °C, the mouse's heart rate soared to 550–600 beats per minute[3]. "When you see, in real time, how animals are responding to [temperature changes]... it led me to say, OK, we really have to pay attention to this variable," says Chawla.

Other researchers are also giving temperature a more serious look. Within the past two years, one study showed that keeping mice at warmer temperatures profoundly changes their physiology, metabolism and inflammation in ways that make them better models of atherosclerosis[4]. Another reported that room temperature induces mild cold stress that speeds bone loss in female mice[5]. And in a third 2016 study, researchers found that mouse models for Alzheimer's developed more

Cold stress can have metabolic consequences: keeping warms means less energy for other functions, says Elizabeth Repasky, who studies temperature and tumors. Image courtesy of Roswell Park Comprehensive Cancer Center.

disease-related pathologies when kept in standard cooler environments[6].

The growing body of studies has piqued the interest of research funding agencies. At the annual meeting of the American Association for Laboratory Animal Science held in Austin last October (2017), the NIH's Office of Research Infrastructure Programs organized a session to explore how temperature and other environmental factors that used to be considered part of husbandry, such as lighting and caging, affect study outcomes. ORIP cares about "rigor and transparency of research, specifically improving the reproducibility of research using disease models," says Stephanie Murphy, director of ORIP's Division of Comparative Medicine. "That's why we're interested in these external extrinsic factors—they relate to rigor and reproducibility."

And when basic research is reproducible, it's more reliable and likely to improve human health, says Brianna Gaskill, an assistant professor of animal sciences at Purdue University in West Lafayette, Indiana, who researches welfare assessment of laboratory animals. If "you're trying to draw information from rodents to help humans, and there are stressors in that animal's environment… it's not a very good model."

As more researchers recognize the importance of ambient temperature on physiological responses in their experimental models, new approaches for maintaining thermal comfort are emerging.

Restoring comfort

Ways to relieve cold stress for lab mice without leaving staff sweltering range widely in cost and labor. Easiest and cheapest: Put more mice in each cage. In a 2014 study, researchers at The Jackson Laboratory, a nonprofit in Bar Harbor, Maine, conducted an experiment to test this simple idea. They placed five strains of mice into standard "shoebox" (78 sq. inch) cages—raising some under near-normal conditions (6 per cage) while housing others more tightly (up to 16 per cage). Every 2–4 weeks over a 3- or 8-month period, the researchers evaluated the mice on mortality, blood cholesterol, immune cell counts, body weight, bone density and behavioral tests—27 features in all. On all but five measures, mice in spacious versus cozy conditions were virtually indistinguishable[7]. Densely housed mice had moderately smaller adrenal glands and lower heart rates. They ate and pooped less. And they had a little more body fat—perhaps from calorie expenditure conserved with extra huddling in cozy conditions, the authors speculate. The housing-related changes fell within physiological ranges, and were small compared to normal variation among strains.

"Some strains will never get along at any density (eg, FVB mice), but for most we can easily add one mouse to six per cage, and we have shown that up to nine in a duplex (51 sq. in.) cage are fine," says Karen Svenson, a Jackson Laboratory research scientist who helped conduct the 2014 study. "We want to use mice in the right way, we want to be nice to them, and we want to get reproducible results," she says.

Another consideration is the dynamic nature of a mouse's "ideal temperature." People are no different. Consider the temperature you prefer when hanging out on the couch watching TV compared to when you're working out at the gym. At the gym you'd "like it a little bit cooler" whereas at home when you're "relaxed, not moving around as much, not creating as much heat… you end up grabbing a blanket to keep yourself warm," Gaskill says. "Basically mice do that."

In a classic experiment done in a breeding cage with tunnels that are heated at one end and cooler at the other, pups wriggled to areas close to their body temperature (37 °C) yet adult mice preferred 30 °C—or even lower if lactating or gestating. "So you have this 10 to 12 degree difference within one cage," Gaskill says. "I think it's a little presumptuous to think we can identify the perfect temperature."

Her suggestion: Let mice create the microclimate they need. In a 2012 study she and coworkers housed mice in a set of two connected cages. One was set to 20 °C and contained varying amounts of nesting material, up to 10 grams. The other was set to one of six temperatures ranging from 20 to 35 °C, but had no nesting material. The mice moved toward the 26–29 °C zone — but if given enough nesting material, they stayed put[8].

Gaskill's team has more recent unpublished data suggesting that the fluff can also act as a stress reducer. When given regular injections of cyclophosphamide—a drug used for chemotherapy in people—mice housed with no nesting material released more stress hormone into their feces. "Having a nest seemed to buffer some of the stresses of being on the toxicology study," Gaskill says.

These days many research facilities routinely equip mouse cages with nesting material, Gaskill says—but often not enough of it. Her studies suggest giving mice at least three full nestlets, about 8–10 grams. "When I give talks, people raise their hands when I ask if they use nesting material, but few keep their hands up when I start asking if they are providing 8–10 grams. It's like me giving you a dish towel to stay warm on a cold winter's night."

Loading cages with nesting fluff sounds simple and reasonably cheap— and it is, at 3 to 6 cents per cage change—and the mice themselves get an element of behavioral control. But for diseases with phenotypes that change drastically in cooler or warmer conditions, scientists may need to adjust temperatures more precisely.

However, it's usually not possible to simply crank up the heat. With many buildings, "air comes in, gets cleaned up and then sent out to the vivarium," Chawla says. "We cannot set the temperature in any particular room because that's central air." Instead, his department uses two-door, stainless steel chambers. Costing $15,000 apiece, each has shelves and maintains a set temperature, humidity and light cycle. With adjustable settings, such special chambers allow researchers to carefully set and control the environmental variables their mice experience.

Labs that cannot afford specialized commercial equipment may not need to suffer sticker shock to gain better temperature control. To help offset the cost of environmental chambers, Repasky is working with Gordon and others on a do-it-yourself strategy. The idea came from a 2009

The temperature may not always need to be increased, but it's a variable researchers should pay attention to, says immunologist Ajay Chawla.

Image: Ajay Chawla, University of California San Francisco

Adding mice could help a cage feel warmer says Karen Svenson; she is studying the physiological effects of housing density.

Image: The Jackson Laboratory

study that examined thermal preferences of mice by heating cage floors with off-the-shelf chemical hand warmers[9]. In those experiments, the researchers simply placed mouse cages on top of the hand warmers. Gordon's team built something a bit more sophisticated: a raised floor with a section cut out to insert an aluminum plate heated by hand warmers. The system maintains a warm 30–32 °C temperature on the plate for about a half day, and in initial tests mice spent more time huddling on heated plates than on non-heated ones in the same cage[10].

At this point it's just a prototype. Since the hand warmers start to fade after about 10 hours, using the system requires daily changes of the heating pad—"a big pain in the neck," says Repasky. Eventually the team hopes to interest mouse cage designers "to do this with an electrical system… to design something that could be turned on at night and off during the day, or vice versa. I don't expect the heat pad model to be a permanent solution."

To warm or not to warm?

Whatever the method, researchers first need to decide whether to relieve cold stress at all—and that choice depends on what questions the experiment will address. "If you want to understand biological programs normally in the mouse... then it's really important," says Chawla.

But in other situations—for example, if you are testing a drug that promotes immune killing of tumors—"you may actually need to house the mouse under thermal stress to compromise immunity to be able to see how to manipulate the pathway to get an effect."

A first step would be to make sure researchers are reporting their mouse housing temperature, Repasky says. Current regulations allow mice to be housed at 20–26 °C. "But a difference of six degrees could contribute to my data saying one thing and someone else's saying something else. If we at least had an idea what temperature an experiment was done at, we might be able to better compare results."

Ultimately, it's not just about temperature. As Repasky's team has discovered, thermal discomfort is a stress that is mediated through similar pathways that mediate other stresses such as anxiety or fear or depression. So putting time and work into creating a warmer environment for lab animals could yield surprising insight into how health and homeostasis go awry in human disease. ❒

Esther Landhuis

Esther Landhuis is a freelance science journalist in the San Francisco Bay Area, San Francisco, CA, USA.
e-mail: *elandhuis@gmail.com*

Published online: 23 April 2018
https://doi.org/10.1038/s41684-018-0044-5

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