JOHNS HOPKINS Magazine

Of Mice and Medicine

Without *Mus musculus* — the mighty house mouse — research at Johns Hopkins could stumble, rather than scurry, along.

By Michael Anft
Illustration by Bill Cigliano

Five days a week, Charles "Chip" Hawkins plays God. He peers down into a microscope and with his left hand twists a lever that holds a mouse embryo in place. With his right hand, he squeezes another lever, this one controlling a one-micron-wide pipette that pokes the embryo, the product of a coupling 12 hours ago. The sphere bunches up before yielding to the pipette's prick, which injects a few hundred strands of DNA into one half of the budding nucleus. The embryo relaxes, as if instantly coming to terms with this transformation of its nature, and returns to being perfectly round.

Hawkins pushes it to the side, uses a suction device to grab another, and repeats the process — up to 10 or so injections a minute, 600 a day. Hawkins, manager of the Johns Hopkins School of Medicine's Transgenic Core Laboratory, and his staff of two spend weekdays

nestled in a stone-silent room high up and deep inside a maze-like building on the Johns Hopkins medical campus, fulfilling 125 or so requests each year from Hopkins researchers to create mice that will become the living tests of their hypotheses.

Hawkins and crew will make transgenic mice — mice with one gene specifically added to make them useful in narrow areas of research — like the ones Hawkins injects now for the lab of Michael K. Lee, a neuropathologist at the School of Medicine. Lee will use the mice to study Parkinson's disease. Hawkins' group might also make a mouse from stem cells taken from a mouse embryo, then grown in a lab and injected with DNA that will switch off a specific gene. The modified stem cells are then transferred to a new embryo to be carried by a surrogate mother, who will give birth to mice that can be bred with "normal" ones to create a so-called knockout mouse (because the gene was "knocked out") so researchers can deduce that gene's function.

"In 25 years, 80 percent of the discoveries we'll see will be traced directly back to the research mouse," says Pardoll.

Once he's done, Hawkins will use a siphon to suck healthy embryos — the ones not turning pale, which means they're dying — into a pipette, and then move them into a dish no bigger than a quarter. Later in the day, the transgenic staff will implant them in female mice that have made whoopie with vasectomized males, so their bodies will release hormones. "The females have to think there is a reason for them to be pregnant for this to work," Hawkins says. In 19 or so days, the new mice will be born, eventually growing to around 20 grams. The cost: about $3,500 per mouse.

But one fully grown mouse is a mere blip on the continuum of what has become a mouse-making industry. "With mice, you spend several years breeding and figuring out what your transgenic mouse is about and what it can do," says Hawkins. Genetically enhanced mice can always be bred with others to make different — and better — research subjects. "Researchers will spend more time to make sure they haven't inadvertently affected a gene they didn't want to affect. Making the right mouse is an ongoing process."

Welcome to the Cult of the Mouse.

Between 20 million and 30 million mice are used nationally each year in biological and medical research, dwarfing the numbers of other animals, including 40,000 monkeys, used to further the aims of science annually. The federal government, as well as university and other labs, "make" many of those millions of mice specifically for a purpose. The world's leading mouse factory, the Jackson Laboratory in Bar Harbor, Maine, breeds 3,500 specialized strains of mice, freezes their embryos and sperm, devises new ways to implant genes, and provides mice to virtually every leading research institution in the United States, including Hopkins. Its 2007 revenue: $150 million.

Last year, Jackson shipped 2.5 million mice around the world. All of its rodents descend from a handful collected 100 years ago by the company's founder, a scientist and university president named Clarence Cook "C. C." Little — either the George Washington or Pied Piper of the modern scientific research mouse, depending on how you look at it (see "A Tiny History of a Wee Research Tool," page 36).

Humans, who have long celebrated clever ways to kill the skittish-but-intrusive rodent, have now cast their lot with it. Rather than build a better mousetrap, we're trying to build a better mouse. Ever since scientists learned how to manipulate a mouse's genes in the 1980s — making it even more suitable for ever-more specific research — the house mouse, or *Mus musculus*, has become a scientific powerhouse. The sequencing of the mouse genome six years ago made the mouse a seemingly endless tool for scientific exploration.

"In 25 years, 80 percent of the discoveries we'll see will be traced directly back to the research mouse," says Drew Pardoll, a professor of oncology at the School of Medicine and a leading researcher on the interrelationship between the immune system and cancer. "The past 25 years have just been incredible. The pace and breadth of research has just exploded — I mean, like times 100. The mouse has always been big. It's just gotten progressively bigger."

Inbred mice have been key. The standard issue of the mouse industry — the eight basic strains of inbred mice — are cheaply produced (unlike their genetically rejiggered cousins) and have the advantage of predictability: Like twins, they carry the same exact genes. "The beauty of inbred mice is that because each one is a carbon copy of another, you can make better comparative studies," says Pardoll. "You can give five mice one type of therapy, and five others another type, and see which is more effective."

Pardoll uses his colony of 3,000 mice to develop and test drug therapies — as has been done around the world for decades — because they have several things in common with humans. They're mammals like us. They have immune systems that are 98 percent identical to ours and genetic make-ups that are about 95 percent similar. In other words, they translate well to human aims, such as the drive to cure cancer.

"The mouse has provided us with an excellent way to model cancer in a mammalian system," says Pardoll. "Every single insight into how the cancer cell and the immune system interact comes from research on the mouse."

Keyata Thompson with her murine charges in the Ross Research Building.

In the past, such insights were achieved through implanting human tumor cells in "nude mice" — hairless rodents with unhealthy or knocked-out immune systems. Tumors grew freely in nude mice, and researchers could easily monitor the growth. But in the past seven years or so, Pardoll says, mice have been genetically engineered to grow their own tumors, which makes them better subjects for research work and clinical studies that can more precisely approximate the development of human malignancies. For example, mice bred to develop prostate tumors have allowed scientists to study a specific immune system response to cancer cells created from their own cells, from within the same biological system. Scientists have been able to devise ways to combine antibodies and vaccines to fight breast and pancreatic cancer and multiple myeloma in mice, and are now translating that work into human clinical trials.

Hopkins researchers from other medical specialties have made gains by exploiting the species' genome to genetically engineer mice to help scientists determine the series of genes that cause Down syndrome. They've made several types of mice that mimic human schizophrenia. They've tested drugs and compounds created to prevent skin cancer, cure malaria, and reverse a potentially fatal weakening of arteries in patients with Marfan syndrome. Several neurologists and brain scientists oversee a one-of-a-kind lab at the Homewood campus that develops and tests mice for a wide range of behaviors, enabling researchers nationwide to investigate the causes and progression of Alzheimer's disease, brain aging, depression, and other conditions — a brave new world of mouse-centric research.

Mice aren't perfect stand-ins for humans — for instance, they don't have the same parts of the frontal cortex that humans do, says Mikhail Pletnikov, an associate professor of psychiatry and behavioral sciences at Hopkins and one of the creators of the DISC-1 mouse, a murine analog for human schizophrenia. ("Murine" is a term meaning "of the mouse.") But, Pletnikov says, mice have become the research subject of choice for scientists studying the brain, especially during the last five years. "If you have a gene, you can make a mouse," he says. "It's a tool that scientists just have to use."

The march of science has often been launched by big minds

unlocking the secrets of small things. Consider Democritus and the atom, Leeuwenhoek and the protozoa, Pasteur and the bacterium, Mendel and the gene.

In an allegedly ventilated, cage-lined cinder-block room in the Ross Research Building, the small things are hardly secretive. The smell — a potent mix of animal dander, feces, and urine — is overpowering. "Most people can't stand it, but I love it," says Keyata Thompson, a research technologist for the School of Medicine's animal research resources department. "It feels like home."

Thompson is one of about 90 people, including seven veterinarians, who care for 200,000 or so mice at 10 facilities across Hopkins. The mice cost the university about 75 percent of its annual $10 million animal care budget — about 74 cents per day for each cage, an average of about a quarter a mouse. (Cages typically can accommodate five mice, but the Hopkins average is three.) Why so many mice? Because they're cheap (about $2 to $3 for a standard inbred mouse), manageable, portable, small, and quickly bred. Using a single inbred line of mice — ones created from a pair whose DNA has been specifically altered by human hands — a viable cancer study can take less than a year. With larger mammals, the timeline grows exponentially.

Nearly a decade ago, 468 Hopkins faculty members kept 42,000 mice. Then things got crazy. "When I got here six years ago, we were seriously overcrowded," says Julie Watson, an assistant professor of molecular and comparative pathobiology and the director of Hopkins' rodent medicine and surgery program. "We've built new homes and taken several new precautions to accommodate the mouse."

Because mice may carry germs and parasites, they can fall prey to many illnesses — bad for the mice and potentially for the caretakers. What's more, murine ailments can also be bad for science. Reviewers of journal articles will ask a researcher if his or her mice suffered from health problems that could skew test results and, hence, a paper's conclusions. "It's a question most researchers really don't like to deal with," Thompson says.

Several years ago, when Watson and leaders of the university's animal research and resources department realized the medical campus was being overrun by mice, they looked with glee at the prospect of moving thousands of them from rooms crammed with cages in older buildings into a brand-new, state-of-the-art vivarium in the basement of the Broadway Research Building (BRB), then under construction. Too many mice were infected with disease-causing pathogens, so Watson saw the new facility as an opportunity to improve the quality of research. "Infected colonies were infecting clean ones, so we needed to come up with a plan," Watson says. "We told researchers at all sites that they needed to clean up their mice if they wanted to use this nice new facility."

Watson devised a program that took mouse pups away from mothers

who may be infected or infested. Investigators who agreed to drop off their pups to Thompson and other animal workers within 48 hours of birth for disinfecting treatments wouldn't be charged for it. In return, they could get their mice into BRB. Researchers bought into the idea.

Now, besides making sure that mice at the medical campus get plenty of rodent chow* — and guaranteeing murine safety and "survivability" by demanding that visitors and researchers wear head and shoe coverings and smocks, and keep their perfume in the bottle at home — Thompson also runs a production colony where she breeds 10 to 20 mice per week. Because pathogens and worms can be passed from generation to generation, Thompson takes those mice from their mothers and cross-fosters them with "clean," or germ-free, mothers. A so-called FBB mouse specially bred to be incredibly maternal will wean mice of, for example, the breed C57/black 6, a hardy staple of a wide range of medical research.

A specially made mouse can mimic a purebred dog. "They're expensive and often temperamental," Thompson says.

Before the pups are placed with their foster moms, they are disinfected. Thompson uses chopsticks to remove the grape-sized pups from their cages, places them in coffee filters so they're easier to handle, then dunks the filter into iodine for a few seconds. "It's nowhere near long enough to drown them," she says. (Before Watson developed this technique, standard procedures included Caesarian sections and embryo transfers, which sacrificed the mothers.)

When clean animals are moved to the BRB basement, they take what Thompson calls "an animal transfer route" underneath the medical campus. "People would freak out if you walked through a facility with an animal," she explains.

You don't have to be anywhere near one of the 19 gleaming "suites," each with five rooms, to sense that there are as many as 100,000 mice in some 26,000 cages at BRB. All you have to do is get on the elevator to experience the waft of murine, um, essence. For humane reasons, mice are monitored daily, with round-the-clock veterinary care for the sick. At BRB and other facilities, mice enjoy fresh pumped-in air, nesting materials that look like a towhead's matted hair, corncob bedding, subdued lighting, and unlimited feed. Robots douse and swab the cages every two weeks, scrubbing away infections and worms. Stickers that read OVERCROWDED are plastered on cages with more than five mice, and researchers who don't fix the problem are charged a daily fine. Of course, for all that comfort, a mouse's lifespan — typically one to two years — might still be shortened by research.

The university took some public relations hits in 2000, when it was

the lone research institution to lobby successfully against more stringent federal regulations targeted at university labs regarding mouse care. The university argued that new rules, which would have required research labs across the country to maintain detailed paperwork on each individual mouse, would cost Hopkins millions of dollars a year. So far, scientists who sacrifice mice in the name of saving human lives have yet to draw the same ire from animal rights activists as have fur-wearers, meat-eaters, and vivisectionists. Most of the complaints Hopkins receives regarding animal care have to do with the School of Medicine's decision to continue to use live pigs as surrogates during surgery. But there are still worries. In February, extremists attacked the husband of a breast cancer researcher at the University of California at Santa Cruz because of his wife's use of lab mice. As the use of larger mammals in research has waned, often replaced by mice, activists have begun to take notice, filling blogs with anecdotal instances of mouse mistreatment and pointing to research that shows that as much as 95 percent of all medical research is done using mice or rats.

Hopkins researchers have developed more sensitive ways to handle mice — and to avoid using them. Alan Goldberg, director of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), notes how imaging devices that use nanotechnology can show researchers how tumors develop and how therapies attack them, without sacrificing the life of the mouse to an autopsy. Much more research is being done in test tubes instead of in living organisms, and there will likely be more if federal strictures against human stem cell research are loosened. Computer models in some cases are replacing the mighty mouse. And scientists have increased the numbers of non-mammalian species used in basic biological and genetic research (good news for mice; not so good for fruit flies and zebrafish).

Hopkins researchers have adopted many of those practices, Goldberg says, but let's be clear: The outcome isn't always pleasant for animals born into the service of humankind. At some research institutions, more than 70 percent of male mice are killed before weaning. That's not true at Hopkins, but males that do not possess the right genes for experimentation often are killed. That's the nature of the research business.

The mouse's enduring utility as a research tool, even with burgeoning alternatives, is a major reason for doing everything to keep a mouse healthy. That, and price. While basic inbred mice cost a couple of bucks, a knockout or other genetically modified mouse can run in the tens of thousands of dollars. In rare cases, an engineered mouse can cost $100,000. An expensive mouse can temporarily disappear into the wrong cage, causing a panic among investigators until it is found and put back in its place. There are other issues for handlers: A specially made mouse can mimic a purebred dog. "They're the ones we usually don't like to work with," Thompson says. "They're expensive and often very temperamental." Apparently, a mouse of high station can bite more.

Sometimes, the mice provide surprises, like the batch that kept dying despite Thompson's care. The scientist hadn't told her that the mice had been bred to have cardiac problems. "I was mortified — I thought it was all my fault," she says as she handles a mouse by the scruff of its neck; the animal scrunches its eyes and makes noises that can only be described as tiny.

Every six months or so, a group of Hopkins pathologists, researchers, and vets holds a conference that could be called "So, We Got This Mouse. What Can We Do With It?" The Phenocore Symposium is designed by vets to answer questions about phenotyping — deciphering what value a mouse has to science.

A phenotype is any observable characteristic that you can measure, anything that distinguishes a mouse type. The basic research mouse, like the black 6, may be better for some experiments than others. Phenotyping can help determine where it can be of most use. Even some transgenic mice have identity issues. Researchers often aren't 100 percent sure how experiments should be designed around a certain mouse, or why, in the worst-case scenario, a mouse died — was it the experiment's design, or some unforeseen genetic complication? At meetings and conferences, Hopkins researchers from a wide variety of specialties compare notes, offering tips on which mice can be used for what.

Although genetics is key in phenotyping, environment can also affect a mouse's viability as a research subject. During a conference held at the medical campus in February, Ellen J. Hess, associate professor of neurology and neuroscience, recalled one group of mice that maintained consistent activity levels in the lab all day long, then quieted down at night, as is typical. But at around 2 a.m. they went into a tizzy. Researchers were baffled by this until a grad student slept over one night and discovered that, precisely at 2 a.m., an investigator in the lab next door cranked up his boom box, setting the mice to dancing.

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At the conference, speakers discussed blind mice, deaf mice, mute mice, mice that "barber" the whiskers off of other mice, and mice that died because they had been implanted with human Alzheimer's genes and couldn't figure out how to use a new water-delivery system in their cages. The moral: Know thy mouse.

The conference also afforded a look inside the industry that has sprung up to handle the mouse once it has been bred, raised, and readied for research. On display were six-lane treadmills, an assortment of mazes, a programmable animal shocker, an eyeblink conditioning system, and the Roto-Rod™ " — a rotisserie-like device that turns slowly in mid-air. Mice perch on the rod and turn along with it, or fall off, indicating whether they are viable for some types of research. At the dozen or so exhibition tables set up outside Turner

Auditorium could be found the Vevo 770 Micro-Ultrasound System for housing and testing mice — winner of a 2007 "Best Customer Value" award. The 12-chamber CLAMS — for comprehensive lab animal monitoring system — was touted by its maker, Columbus Instruments, for keeping track of how much the mice inside it have consumed and slept, their body mass, ambient light and room temperature, their heart rate and temperature, and other measurements. "They start at $50,000," says Ken Kober, the company's sales manager, "and then they start to get expensive. I've seen them go for as much as $90,000. The Big Pharma guys love them."

Across the city, at the Neurogenetics and Behavior Center at Mudd Hall on the Homewood campus, even more specialized machines — including odor discrimination equipment and single-trial aversive learning cages — fill a basement lab/vivarium. Here, researchers phenotype mice for the benefit of researchers worldwide. The five-year-old center has 40 Hopkins faculty members and several outside partners, including the Jackson Laboratory and the Howard Hughes Medical Institute. Backed by millions of dollars in grants from the National Institutes of Health, the center will put mice through specially designed paces to see what they might bring to the brain-research table.

"We're trying to find the best ways to perform research on the human brain using mice," says Michela Gallagher, vice provost for academic affairs and a professor of psychology and neuroscience at the Krieger School of Arts and Sciences. "Do they function best in large groups? Will they behave differently if they're removed from a group? We're ground zero for getting those types of behavioral measures out of mice."

The goal is to find ways to compare mouse and human behavior, so that aging diseases like Alzheimer's and mental illnesses like depression and schizophrenia can be studied in rodents. For decades, psychologists have preferred rats, often creating lesions in the animals' brains to see how the lesions affected behavior. "Mice do OK in our experiments, but they're not brilliant — rats have been smarter," says Gallagher.

Yet, even here, in the one-time domain of King Rat, the mouse reigns supreme. Because of its genetic malleability, the mouse has become the animal of choice for the center's faculty studying brain disorders. Instead of implanting lesions, or even genes, in rats, researchers have made transgenic mice their instrument of choice because of their availability, and Hopkins researchers' ability to create and rapidly breed them.

Research technologists disinfect grape-sized

mouse pups, then place them with foster mothers.

The pre-eminence of mice has changed brain science. "The mouse represents a new phase in my research," says Pletnikov, a member of the Neurogenetics and Behavior Center. "I've had to learn how to do genetic models — to insert DNA into a mouse genome. Previously, I concentrated entirely on how viruses and other environmental factors affect the brain. Now I'm also investigating how proteins and receptors — the molecular pathways that can affect brain health — are regulated by genes." Mice implanted with the DISC-1 gene show the same hyperactivity and abnormalities in social behaviors as humans who suffer from the disease — a sign that they have validity as research tools for investigating schizophrenia in humans, Pletnikov says.

Disorders such as schizophrenia are present in a person's genes before birth, but only manifest themselves after the onset of adulthood. Mice can add an extra dimension for researchers because of recent discoveries that allow for controlling a gene's expression with the help of pharmacology. For example, researchers can "turn off" a gene or delay its expression by giving a young mouse a simple drug in its food (in this case, the common antibiotic tetracycline). The drug binds to a protein that prevents the protein from binding to another one upstream, an event along a so-called molecular pathway that would activate the schizophrenia gene. Once the drug is removed, the adult mouse will demonstrate the murine equivalent of human schizophrenic behavior. Because the mouse can be both a disease carrier and yet not exhibit behavior of the disease, it accurately approximates the manifestation of schizophrenia in people. The mouse also performs double duty for researchers because they can see how the gene acts when it is on or off. "The control is built right into the mouse," Pletnikov says.

In the basement at Mudd, Gallagher and her crew study aging, autism, eating and obesity, learning, and memory in mice while searching for ways to compare mouse behavior with that of humans who suffer from disease. For example, Gallagher is working to model the onset of Alzheimer's. Brain circuits in rodents with the disease that govern their spatial memory appear to break down early — something that can be extrapolated to future studies of human subjects, she says.

The potential for so-called translational work is what drives the center, says Alex Johnson, a post-doctoral fellow in psychological and brain sciences. "We're doing things with mouse genetics that no one else is doing," he says.

Science has not yet exhausted the uses of the humble mouse — far from it. For example, as good as mice have been for his cancer studies, Pardoll says the future might be brighter. The advent of the "humanized mouse" — one that carries a match of the tumor and the blood system, including the immune system, of a particular patient — is on the horizon. That may lead to the ultimate mouse model: one unique to an individual person's biology. Research so far is promising, but is hung up on two questions: Will the tissue of a mouse reject the human immune system? And, human blood travels through arteries, capillaries, and vessels, but unlike in a mouse's system, it also flows through certain cell membranes. Will a mouse's biology be able to handle it? "It all seems possible, but doesn't quite add up yet," says Pardoll. "We're many years away from answering those questions."

Such research will continue as the prospects of the mouse skitter off in some bold, often opposite directions. The National Institutes of Health is pouring $60 million into a "knockout mouse project" to map the function of every known mouse gene to aid the cause of ever-more-specific research. At the same time, the federal government and the Jackson Laboratory are breeding 1,000 new and genetically mixed mouse strains from the original eight basic inbred ones. A more varied population of mice than the descendants of C. C. Little's should create genetic diversity — all the better for them to represent the diversity of humans in experiments. Some scientists blame faulty — and ultimately deadly — clinical trials involving such drugs as Vioxx and Phen-fen on the fact that they were initially performed on mice with the same handful of genes, yielding poor translational results. Others argue that medical research hasn't gone as far as it could because the mice and their gene pools haven't been up to it.

Another school of thought believes that mice confined in dark silence do not develop fully, further limiting their suitability as research subjects. A mouse's surroundings, the thinking goes, should be made more stimulating, so their brains and nervous systems can flourish, or more closely approximate what they would become in nature. "Geneticists have thought of the mouse as a furry test tube," says Cory M. Brayton, an associate professor of comparative and molecular pathobiology and director of the university's phenotyping program. "You drop your mutated gene in there and you should find out specifically what it does. They forget that the mouse is an animal with a biological system."

Such thoughts don't veer all that close to anthropomorphism, but they do show that an animal drafted as a stand-in for humans might just be worthy of a morsel of respect.

Michael Anft is a senior writer for Johns Hopkins Magazine.

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* This corrects earlier information published in the September issue print edition.

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The Johns Hopkins Magazine | 901 S. Bond St. | Suite 540 | Baltimore, MD 21231
Phone 443-287-9900 | Fax 443-287-9898 | E-mail jhmagazine@jhu.edu