Gleaning Meaning From Ailing Mice

Bedridden lab animals reveal details about human diseases

By RICK WEISS

Until recently, any mouse at the Jackson Laboratory showing even a hint of intestinal disease got a one-way ticket to Animal Health, the department that disposes of sick animals at this giant mouse-breeding facility in Bar Harbor, Maine. Now the same animals — many with diarrhea and other signs of intestinal infection suggesting parasites or *Salmonella* bacteria — get sent down the hall to geneticist Edward H. Birkenmeier.

In taking the time to care for rodents formerly relegated to the crematorium, Birkenmeier might seem a sort of Mother Theresa for mice. But his motivation doesn't come from a desire to save mouse lives. His goal is to find cures for human diseases.

Birkenmeier has evidence that some of the lab's sick mice are not infected at all. Rather, they seem to harbor specific genetic defects that leave them with infection-mimicking digestive and metabolic disorders. Moreover, many appear to have inherited syndromes closely resembling certain hereditary human diseases. Birkenmeier nurtures and breeds these unique strains of mice, and has recently begun using them to test new drugs and genetic therapies that may someday prove useful in humans.

His work is part of a rapidly growing research specialty that has scientists collecting — and in some cases creating — mice bearing essentially human genetic defects. Already, researchers in this field have developed mouse models for such diverse diseases as sickle cell anemia, epilepsy, diabetes and AIDS. But while inherited digestive defects are relatively common in humans, well-characterized mouse versions remain rare. For instance, Birkenmeier says, "there are no well-defined mouse models for colitis and related human intestinal diseases."

Jackson Laboratory ranks as the world's largest mouse-breeding facility, distributing more than 2 million custom-bred mice to research scientists every year. Given those numbers, Birkenmeier reckons that several mutants with rodent-equivalents of human inherited intestinal disorders have been born there over the years. But scientists at the lab, fearing an epidemic of the "rodent runs" the way a match manufacturer might worry about a fire, haven't held on to these animals long enough to perform detailed genetic analyses. Without a concerted effort to cull digestive-tract mutants from truly infectious mice, many valuable strains have probably been discarded, Birkenmeier says.

That's a trend he's trying to reverse.

Jackson Laboratory's new commitment to examining seemingly infectious mice stems in part from recent experiments Birkenmeier performed on progeny of a mystery mouse born there about 15 years ago. That dwarf mouse featured skeletal abnormalities and atypical fat deposits, but no infection. At first, scientists at the lab thought the runt might be suffering from a diminished supply of a growth hormone. Later, they suspected a defect of the pituitary or adrenal glands. But all tests came up negative.

Eventually, researchers routed the mouse to Birkenmeier, a specialist in lipid metabolism. He studied the animal for two years before determining that it had a genetic disease called mucopolysaccharidosis (MPS) — one of several lysosomal-storage disorders, which affect the tiny digestive sacs, or lysosomes, inside many types of cells. In humans, clinicians have identified seven major classes of mucopolysaccharidoses. This was the first case seen in a mouse.

Detailed studies indicated the mouse failed to produce sufficient quantities of the enzyme beta-glucuronidase, which normally helps break down metabolic by-products called glycosaminoglycans. The resulting accumulation of these by-products poisons the cells, causing growth deformities and, in many cases, premature death.

Birkenmeier immediately recognized the potential importance of his discovery. In humans the same disease, known as mucopolysaccharidosis type VII (MPS VII), remains largely incurable. Bone marrow transplants have shown some promise, but they do not guarantee success, and in some cases prove fatal.

In theory, periodic intravenous replacement of the lacking enzyme might

Of mice and modems

In the past few years, geneticists' interest in mouse DNA sequences — which closely resemble human DNA sequences in some disease-related regions — has grown into a full-scale attempt to map every gene on the mouse's 20 pairs of chromosomes.

This international project has already generated computer disks full of information about specific mouse genes and mouse diseases. A parallel endeavor to map the entire human genome has produced similar quantities of genetic data. But researchers have remained hobbled in their attempts to match equivalent genes in mice and humans, both because of the different computer languages used by different research teams and because the major databases have lacked direct electronic links.

This fall, those problems should begin to dissolve. A novel computer network — a joint effort of the Jackson Laboratory in Bar Harbor, Maine, and the Johns Hopkins University School of Medicine in Baltimore — will open new avenues of information-sharing between researchers exploring human and mouse genes. Ultimately, it may fill critical gaps in scientists' understanding of the 3,000 or more genetic defects that cause human disease.

When complete, the computer project will direct cross-communication among four bodies of information: the Genomic Database (GBASE), a collection of mouse-gene locations; the Mouse Locus Catalog (MLC), which outlines the genetics behind mouse diseases; the Genome Data Base (GDB), a catalog of human genes; and Online Mendelian Inheritance in Man (OMIM), a computerized map showing the chromosomal locations of human disease-causing genes.

Already, GBASE and OMIM are on line — available around the world to scientists whose computers are equipped with telephone hookups, or modems. GDB will come on line for the first time in September, Hopkins recently announced. And MLC should become integrated with the network within about a year, says Tom Roderick of the Jackson Laboratory.

At that point, the system should be completely "transparent," allowing researchers to "float easily between all these databases," Roderick says. Scientists "will be able to call up a mouse gene and see if there is a human [equivalent], or vice versa, and compare the clinical manifestations of both." — *R. Weiss*

benefit MPS VII victims. But until the discovery of the MPS mouse, scientists had no idea which of several chemical forms of the missing enzyme might work, or how much to give. Birkenmeier described some of the latest experiments with these mice at a course in medical and experimental genetics held at the Jackson Laboratory last month.

In one set of experiments, he gave MPS VII mice intravenous doses of human beta-glucuronidase provided by John W. Kyle and William S. Sly at St. Louis (Mo.) University School of Medicine. They had found that by inserting the human beta-glucuronidase gene into bacteria, they could induce the bacteria to make a "raw" version of the human enzyme. But when they inserted the same gene into cultured mouse cells, which are biochemically more sophisticated than bacterial cells, the cells not only manufactured substantial quantities of the enzyme but also processed it in ways that made it more active. It was this form of the enzyme that Birkenmeier injected into his sick mice, and with stunning results.

"The animals are amazingly sensitive to the injections," Birkenmeier says. Even very small doses of the enzyme apparently correct the metabolic defect, reversing some symptoms and, if given early enough, blocking disease progression. He anticipates that ongoing mouse studies will culminate in clinical trials of an intravenous beta-glucuronidase treatment for children.

The benefits of early intravenous beta-glucuronidase supplements might not have seemed so exciting a few years ago, Birkenmeier says. Until recently, physicians had no way to identify MPS VII children before glycosaminoglycans accumulated with irreversible effects. But a new test, developed by Chester B. Whitley and his colleagues at the University of Minnesota in Minneapolis, appears capable of making this early diagnosis. The test, described in the February 1989 CLINICAL CHEMISTRY, assays glycosaminoglycan levels in urine. Those levels are elevated in newborns with mucopolysaccharidoses such as MPS VII, tipping off physicians to the impending problem.

Beyond their usefulness for testing conventional therapies, MPS VII mice show promise as test subjects for gene therapy. Kyle and others at the St. Louis University School of Medicine, working with Birkenmeier and his colleagues from the Jackson Laboratory, recently inserted the gene for human beta-glucuronidase into MPS VII mice. These genetically engineered animals, now able to make their own beta-glucuronidase, show no signs of the storage disease. Details of the work appear in the May PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (Vol.87, No.10).

Although gene therapy for humans with MPS VII remains years away, the engineered mice hint at a permanent cure for this and related disorders, Birkenmeier says. He notes that MPS VII is "relatively rare" in humans, "but taken together, lysosomal storage diseases represent a significant clinical problem," especially among certain ethnic groups such as Ashkenazi Jews.

For example, two of the more prevalent lysosomal-storage disorders — Gaucher's disease and Tay Sachs disease — strike one in 600 and one in 3,000 Ashkenazi Jews, respectively, according to figures compiled by geneticist Victor McKusick at the Johns Hopkins University School of Medicine in Baltimore. And while physicians worldwide have diagnosed only 25 to 30 people with MPS VII, one in 70,000 individuals in Israel is born with MPS II, while MPS III affects one in 24,000 in the Netherlands.

Birkenmeier proposes that widespread application of the infant screening test for MPS, followed by intravenous therapy for those testing positive, could go a long way toward preventing onset of the disease. And in the long run, he says, genetic manipulation of patients' cells may provide a permanent cure.

Although his work with MPS mice continues, Birkenmeier has broadened his interests to encompass inborn intestinal disorders. By intercepting ill mice on their way to Animal Health, he hopes to do for these pernicious diseases what he's begun to do for lysosomal-storage diseases.

Already, Birkenmeier has initiated tests on a few candidate mice to see if their intestinal problems have a genetic basis. It may be years before the work leads to useful models of human digestive diseases, he concedes. But he also knows that with perseverence and a little bit of luck, some runted rodent may emerge with the answer to many people's most gut-wrenching problems. □

Continued from p.121

has found that predators riddled the unperforated shells of other animals but left *Mickwitzia* alone.

Mickwitzia and *Mobergella* notwithstanding, paleontologists have only sketchy evidence that early Cambrian skeletons served as predator-resistant armor. Most of the small shelly fossils from that era remain an odd assortment of caps and cones, spicules and scales. "And they're largely enigmatic in that these wretched things fell into thousands of bits when they died," says Conway Morris, who likens the reconstructor's task to throwing a jigsaw puzzle out of an airplane and reassembling it on the ground. So far, the shards offer few hints of defensive functions.

Yet as excavations proceed at quarries around the world, new finds strengthen the case for an early Cambrian arms race. From an extraordinary fossil bed discovered in 1984 in north Greenland — predating the Burgess shale by perhaps as much as 15 million years — comes a jigsaw puzzle already assembled: a suspiciously familiar, slug-like beast sheathed in chain-mail armor. In the June 28 NATURE, Conway Morris and John S. Peel of the Geological Survey of Greenland in Copenhagen, Denmark, describe an unprecedented discovery: the complete skeleton of an early Cambrian "halkieriid," which they propose as the long-sought ancestor of the armored slug *Wiwaxia*.

Though the halkieriid lacks *Wiwaxia's* dorsal spines, it sacrifices nothing to strangeness. To the bafflement of its discoverers, the creature sports a disproportionately large, saucer-like shell at each end of its elongated body. Bengtson speculates that these served to plug the entrance to the halkieriid's U-shaped burrow. McMenamin, adding yet another twist, says the posterior shell so resembles the clam-like *Mickwitzia* that he now believes *Mickwitzia* was not an organism unto itself, but rather a piece of armor worn by a larger animal that resembled the halkieriid.

From another recent fossil discovery at a quarry in south China — which appears even older than the Greenland site — emerges the bizarre *Microdictyon*. Unveiled last year by Chinese paleontologists, *Microdictyon* is a wormish creature with a row of pointed appendages and a body studded with oval phosphate plates. Bengtson, who says the animal must have looked "like something out of a bad dream," thinks the plates might have served as some sort of antipredator armament.

Bit by bit, the skeletal puzzle comes together. Conway Morris says about 30 quarries worldwide are beginning to yield Burgess-quality fossils, with many more sites yet to be discovered. And he believes it's only a matter of time before paleontologists track down the original cast of predators that might have helped incite the skeletal stampede. Fossils now show, for instance, that the trilobite-chomping *Anomalocaris* identified at Burgess also roamed the early Cambrian seas.

"When we find the equivalent of the Burgess shale right at the base of the Cambrian period, as surely we will, we will find that there are all sorts of interesting predators," Conway Morris predicts. "Ultimately, I think we're going to be able to integrate the whole thing into quite a nice story." □