Power Failure

What happens when muscle cells run out of fuel

By KATHLEEN FACKELMANN

Imagine a scene roughly one and a half billion years ago. An energy-poor cell floating in the ocean swallows a bacterium that has a talent for making a fuel molecule called ATP. The cell soon recognizes the benefits of an in-house fuel factory. It makes the bug a permanent resident.

Fast-forward to 1997. Deep inside each human cell are hundreds of structures widely believed to be the descendants of that bacterium. Biologists now call them mitochondria, and they literally power most of the activities of human cells.

"We have a colony of bacteria in our cells," says geneticist Douglas C. Wallace of Emory University School of Medicine in Atlanta. "They make energy by burning the food that we eat," he says.

Scientists have long known that people inherit some diseases through defects in the 100,000 or so nuclear genes—DNA located on the 46 chromosomes in each cell's nucleus. Recent studies have shown that some human diseases can be traced to flaws in the mitochondrial genes, the only DNA located outside the nucleus of animal cells.

Nine years ago, Wallace and his colleagues discovered the first inherited mitochondrial DNA defect—a mutation that results in a rare form of blindness called Leber's hereditary optic neuropathy. In 1992, the researchers demonstrated that flaws in mitochondrial DNA can, in rare cases, lead to type 2 diabetes, a disease in which the body can't process sugar properly. By 1995, Wallace's group and several other teams had evidence suggesting that such defects may underlie some cases of Alzheimer's disease and other neurodegenerative disorders (SN: 8/5/95, p. 84).

Now, Wallace's group has found that the introduction of mitochondrial energy defects into mice can also cause heart and muscle disease. This leads Wallace to suspect that mitochondrial malfunctions may trigger some cases of cardiomyopathy, a disease of the heart muscle that afflicts up to 50,000 people in the United States. He and his colleagues have already linked mitochondrial damage to other forms of heart disease (SN: 10/5/91, p. 214).

To understand how the new research came about, one must first consider that ancient cell's encounter with the fuel-generating bacterium. To get any benefit from ATP, the cell had to get it out of the bug. The cell therefore created a protein that ferried ATP through the outer membrane of the bacterium.

The adenine nucleotide translocator (ANT) protein is like the nozzle that transports fuel from a gas station pump to a car. If the nozzle doesn't work, gas can't get to the tank and the car can't move. Likewise, ATP has to be pumped from the mitochondria into the cytoplasm of a cell. The cell uses ATP for a variety of crucial functions. Muscle cells, for example, need this fuel in order to contract.

There are several types of this translocator protein, and they have been found in different tissues. Wallace and his colleagues wondered what would happen if they disabled the gene for ANT1, the protein that skeletal muscle and heart cells use. They reasoned that without the protein, skeletal muscle and heart cells wouldn't have enough energy to contract.

To test their hypothesis, the researchers incapacitated the *ANT1* gene in mice. They then studied the mice for signs of energy deficiency.

First, the team scrutinized skeletal muscle. Samples from the genetically engineered mice revealed ragged muscle fibers with abnormal mitochondria. Wallace notes that people with similar muscle fibers, called ragged-red muscle, are diagnosed with mitochondrial myopathy, a disorder that causes extreme fatigue and an intolerance for exercise.

Closer examination of the samples revealed that the mitochondria had proliferated in the muscle cells, displacing the muscle contraction machinery.

"What we've got is a mitochondrial cancer," Wallace said in July at Press Week 1997, a meeting in Bar Harbor, Maine, sponsored by Jackson Laboratory and Johns Hopkins University. Although mitochondria don't invade other cells, the wild proliferation destroys the structures in the cells they inhabit, Wallace says.

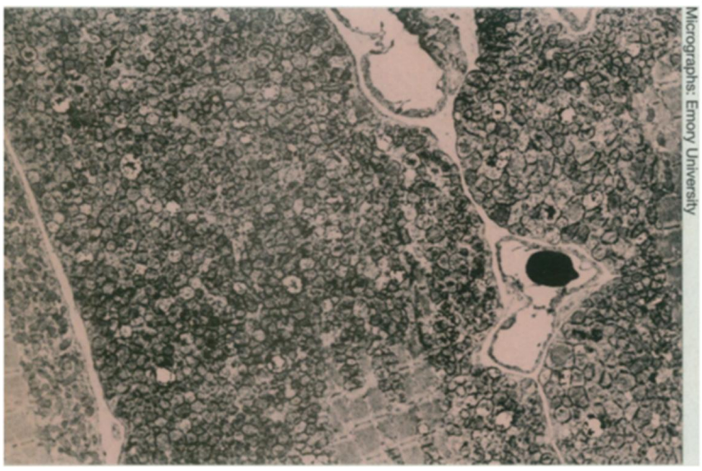
Equally striking, the mitochondria in each muscle cell appeared bloated. These enlarged structures make ATP, but because they lack the ANT1 protein, the fuel can't move into the cytoplasm, where it is needed. Wallace believes the cell commands the mitochondria to divide and enlarge in an ill-fated attempt to compensate for its lack of usable fuel.

Next, the researchers turned their attention to the cardiac muscle. When they looked at 4- to 6-month-old genetically engineered mice, they found the animals' hearts were 50 percent larger than those of ordinary mice.

The researchers also noted that the mutant mice suffered from a thickening of the walls of the heart's left ventricle. When abnormal mitochondria don't turn out enough energy for heart muscle cells to contract as they should, the body may compensate with such anatomical changes, says Wallace. The changes, in turn, can bring about a life-threatening decline in the heart's performance.

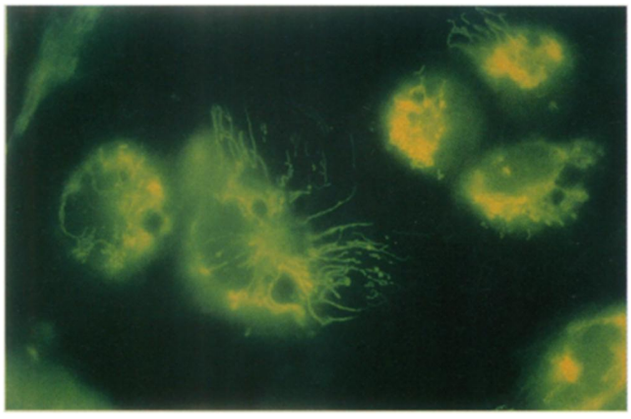
Both enlargement and thickening are symptoms of a type of cardiomyopathy in people.

The team found that the mitochondria in the heart muscle of the mutant mice, like the mitochondria in skeletal muscle, had gone on a dividing spree. However, the mitochondria in heart cells did not appear larger than normal. The researchers speculate that because the heart



Micrographs: Emory University

Colorized electron micrograph shows the proliferation of enlarged mitochondria (small ovals) in genetically engineered mice. Normal skeletal muscle resembles cells at lower left.



Mitochondria in a normal human cell can be stained with a fluorescent yellow dye.

cells also produce ANT2, another form of the adenine nucleotide translocator protein, enough ATP gets out to prevent enlargement of the mitochondria.

People diagnosed with mitochondrial disease often have a defective system of oxidative phosphorylation, the process by which mitochondria make ATP. This flaw shows up as elevated concentrations of organic acids in their blood. Wallace and his colleagues noted that the genetically engineered mice had four times as much of one such acid as normal mice did. Thus, the biochemical profile of the engineered mice appeared consistent with faulty ATP production, says Wallace.

Moreover, people with mitochondrial disease suffer from fatigue and muscular weakness. Eventually, many need help with their daily activities. Wallace's team decided to find out whether the genetically engineered mice also suffered from weakness and fatigue.

The researchers subjected normal and mutant mice to a 25-minute treadmill test. Normal mice had no trouble with this challenge: Even at the highest speed and inclination, they trotted along with no apparent difficulty. In contrast, not one of the genetically engineered mice could complete the test. This finding suggests that the abnormal mitochondria in their skeletal muscle can't keep up with the cells' demands for energy, says Wallace.

Wallace's team published its findings in the July Nature Genetics. Together, the results suggest that a defect in oxidative phosphorylation can result in the skeletal and cardiac muscle symptoms characteristic of mitochondrial disease.

"This is the first time that we can show a cause-and-effect relationship between limiting ATP and the pathophysiology that has been correlated with [mitochondrial] diseases," Wallace said at the Bar Harbor meeting.

Donald R. Johns of Beth Israel Hospital in Boston agrees that Wallace and his group have created the first mouse model of mitochondrial disease, but he adds an important caveat: No human patients have been found with a defect in the *ANT1* gene. Thus, the mouse model does not mirror any known human genetic defect leading to a mitochondrial disease.

"That's a weakness in the work," agrees Eric A. Schon of Columbia University. "But it's not much of a weakness since nobody knows a whole lot of anything in terms of mitochondrial disease."

Wallace points out that no one is ruling out a human version of the *ANT1* defect. It's just that researchers have yet to find such a disease-causing mutation in patients.

The model may prove enlightening in any case—because the mice do exhibit many of the features of human mitochondrial diseases, says Schon. His team and others are working on animal models of mitochondrial disease.

A realistic animal model of mitochondrial disease might help prove or disprove a controversial hypothesis. Some researchers, Wallace included, believe that mutations in mitochondrial DNA account for more than just rare diseases. "Aging itself may be at least in part mediated by an accumulation of mutations in the mitochondrial DNA," Johns says.

In the process of making ATP, mitochondria spew out reactive oxygen molecules called free radicals. Many scientists think free radicals injure the DNA of the mitochondria. As the damage accumulates, the mitochondria have more trouble cranking out ATP. Brain, heart, and muscle cells, which require the most ATP, start to falter.

Older people who notice a lack of zip, says Wallace, may be feeling the effects of the failing power plants in their cells. "I've felt for years that [older people] were telling me verbally what we were seeing in the laboratory," he says. "What really robs old people of dignity is their loss of energy," he continues.

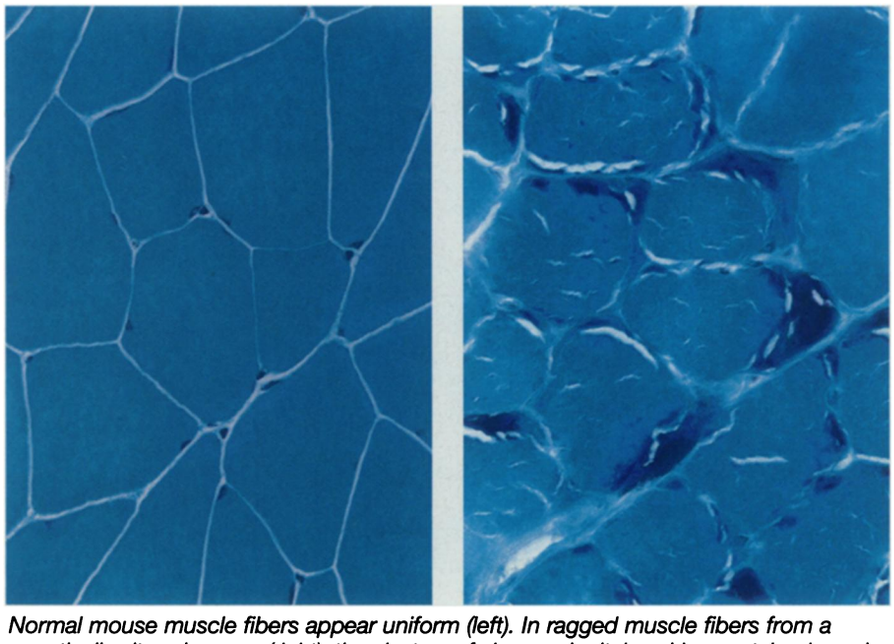
Wallace believes that if they could protect their mitochondria, people might be able to mitigate some of the debilitating effects of old age. Muscle fatigue, heart failure, and memory loss may all stem from mitochondria that can't keep up with the cells' energy requirements.

Other scientists remain cautious about accepting this theory. There's a lot of speculation about free radicals but no proof that these substances actually damage the mitochondrial DNA and lead to the symptoms of aging, says Eric A. Shoubridge, a mitochondria researcher at McGill University's Montreal Neurological Institute. "One would think [free radicals] have a role, but I would like to be convinced," he says.

Wallace and other scientists have demonstrated that animals, including humans, do accumulate mitochondrial mutations as they get older. To say that those mutations cause old age remains a leap—at least for now.

Research on mitochondria won't lead to an antidote to aging. Even if one could fix the mitochondria, some other system would break down in a person approaching the century mark, Wallace points out.

Still, Wallace believes that reversing the energy drain would provide a significant benefit. "We're looking at the mechanism that causes day-by-day degeneration—that's what we want to solve." □



Normal mouse muscle fibers appear uniform (left). In ragged muscle fibers from a genetically altered mouse (right), the clusters of abnormal mitchondria are stained purple.