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Photographs by Addison Geary

What Lee Sweeney discovers about muscles in mice is likely to have a bearing on muscles in humans.

In scientific circles, Lee Sweeney has built a reputation investigating Duchenne muscular dystrophy and hypertrophic cardiomyopathy – but he is best known in the wider world for his work with “mighty mice,” treated to gain muscle mass and stave off many of the effects of aging.

What makes Lee Sweeney run? Muscles. H. Lee Sweeney, Ph.D., chair of Penn’s Department of Physiology, has spent his whole career learning how muscles work at a molecular and cellular level. And what he’s found has revealed new insight not only into what can go wrong in muscles, but also how non-muscle cells communicate and carry out many essential functions.

Along the way, muscles have carried Sweeney into what is usually unknown territory for most basic scientists. He is as likely to be spotted at meetings of politicians, policy makers, ethicists, parents, and athletes as at meetings of his cellular biology colleagues. Yet this should not be surprising, given that dysfunc-

What Makes Lee Sweeney Run?

By Lisa J. Bain



tion of muscles is responsible for so many human diseases and disabilities. Heart disease, the leading killer of people in America, is essentially a disease of a large muscle. And many conditions that bedevil the ever-growing aging population result from loss of muscle mass.

“My intellectual love is the very basic work that I do,” he says, but he concedes that “it would be a stretch to see how the basic work that I do would impact society or the health of society in the next few centuries.” On the other hand, Sweeney also has what he calls his “more applied work” and his work to try to help patient groups that are interested in diseases of the muscles. That activity started, he says, as a means “to do something that would be a little more relevant to the society that supports me in my ability to spend most of my time doing things that I find intellectually challenging.”

Running on molecular motors

Sweeney came to Penn in the late 1980s, at a time when the study of physiology was moving rapidly into the realm of molecular biology. Yale Goldman, M.D. '75, Ph.D., now professor of physiology and director of the Pennsylvania Muscle Institute (P.M.I.), recruited him, recognizing that he had both a strong foundation in physiology and had begun using molecular techniques

to study the problem of muscle contraction in skeletal muscle. Penn and the P.M.I. had a long tradition of excellence in research on muscle physiology, and Goldman rightly sensed that expertise in molecular biology was essential for continuing that tradition.

Goldman and Sweeney took parallel paths to study a group of muscle proteins called myosins. Sweeney focused on molecular aspects, while Goldman undertook biochemical and structural studies. Myosin is called a molecular motor because it transforms chemical energy into movement along tracks made up of another protein called actin. In this way, myosins power the movement of muscle fibers during muscle contraction. But myosins do much more: they are responsible for cellular motion during cell division as well as for the movement of different structures within the cell.

“Lee had a brilliant insight about the tilting motion of one of the subunits,” says Goldman. “A lot of us had been looking for angle changes in myosin, but the evidence was slim.” Sweeney’s work with colleagues at The Scripps Research Institute in La Jolla, Calif., provided some of the most direct evidence that a part of the myosin molecule acts as a rigid lever arm, swinging the myosin down the actin track. Meanwhile, work in Goldman’s lab using a novel technique called fluorescence polarization also helped solidify the idea that a central feature of motility involves a lever-arm motion of myosin.

According to Goldman, Sweeney and he are planning future collaborations studying non-muscle myosins in the brain. These proteins carry cargoes around inside of cells. Sweeney and Amber Wells, who had been a graduate student in Sweeney’s lab (she completed her Ph.D. degree in 2002) discovered a novel feature of one of these myosins, called Myosin VI. Unlike all other known myosins, Myosin VI walks backward on actin, giving rise to novel functional properties.

The discovery, says Wells, “opened up a whole new way to think about how myosin may be acting.” This new concept may help explain how

mutations in the protein can cause deafness in both humans and mice. At least two other myosins have been linked to deafness, pointing to an essential role for the protein in the development of the ear. Mice with a mutation in myosin VI, called Snell’s waltzer mice, also have balance problems, run in circles, and show signs of anxiety, suggesting even further intrigue in the myosin story.

Alternate paths

Within a few years of coming to Penn, Sweeney had made an impres-

sion on other investigators within the University community. Alan Kelly, B.V.Sc., M.R.C.V.S., Ph.D., now dean of the School of Veterinary Medicine, convinced him to apply his knowledge of muscle physiology to a fatal, degenerative disease – muscular dystrophy. Sweeney also initiated studies of myosin contractility in heart muscle, eventually leading to collaborations with Timothy J. Gardner, M.D., now the William Maul Measey Professor of Surgery and chief of cardiac surgery at the Hospital of the University of Pennsylvania. And, as he began to

Sweeney and the Hulk



Sweeney’s mice have shared the spotlight on TV.

In May 2003, the Discovery Channel aired a program called “Kapow! Superhero Science.” The question it examined was “can science replicate the powers of comicbook superheroes?” (For unexplained reasons, all the superheroes cited come from Marvel.) One of the program’s segments focused on strength well beyond normal, as embodied by the Hulk, the massive green creature with an unfortunate tendency to lose his temper. The scientific background featured the work of H. Lee Sweeney, Ph.D., chair of Penn’s Department of Physiology. As the Discovery narrator put it, “It appears that to really change human to Hulk, you’re going to have to tamper with the genes,

which is where the real super-science comes in. Dr. H. Lee Sweeney of the University of Pennsylvania has already created the Hulk – or at least his rodent equivalent.”

Filmed in his laboratory, Sweeney demonstrated one of the mice used in his experiments aimed at slowing the loss of muscle strength. If you shaved off the fur of a treated and an untreated mouse, he said, “there’d be no comparison. I mean, the muscles are huge in the treated ones vs. the untreated ones.”

The narrator filled in some of the details: “The scale tells the tale: a normal mouse weighs in at 27 1/2 grams; the genetically engineered ones, 41 grams – or 40 percent

think more and more about treating these debilitating diseases, Sweeney began collaborating with James M. Wilson, M.D., Ph.D, the John Herr Musser Professor of Research Medicine who headed Penn's Institute for Human Gene Therapy.

Sweeney's early work in muscular dystrophy remained on a basic science level. "My interest in trying to develop something therapeutic didn't come until I was first asked to speak to some of the parent groups," he says. "It was the personal contact that sort of changed my views."

There are actually several forms of

muscular dystrophy. All are inherited, many in an x-linked fashion, meaning they affect only boys and are passed through mothers. Duchenne muscular dystrophy (DMD), the most common and severe form, affects about 1 in every 3,500 male births. Affected boys may appear normal until they begin walking. Sometime between the ages of one and three, they begin to show signs of weakness and may have trouble standing. As the disease progresses, skeletal, respiratory, and cardiac muscle are all destroyed. By age 10, most boys with Duchenne

muscular dystrophy are confined to a wheelchair, and they rarely live past their early 20s.

Sweeney's work with parent groups finds him not only meeting with parents to tell them about progress in the field, but also lobbying Congress for more funds to support muscular dystrophy research. "With the form of muscular dystrophy that I've been working the most with, the patients themselves don't live long enough to lobby Congress," he says. "And by the time they die, most of the parents never want to think about it again. So historically, there has been very little voice for that community in Congress because of the nature of the disease."

Pat Furlong, president of Parent Project Muscular Dystrophy (PPMD), says that Sweeney backs his words with action. At the first PPMD conference he attended, says Furlong, Sweeney sat through the entire conference, not just the scientific sessions. "I was thrilled, pleased, amazed," she says. At one point, "he stood up and said, 'The only way you're going to get sufficient money for us to do something about this disease is to lobby in Congress.'" And that is exactly what Sweeney has done. Says Furlong, "Of all the researchers in the world, I admire him the most because if he promises something, he'll deliver." Sweeney now serves as the group's scientific director.

Duchenne muscular dystrophy is caused by mutations in the gene for dystrophin, a protein that is needed for the structural support of muscles. Without dystrophin, muscles deteriorate and weaken. Although some early work suggested that gene therapy might be used to replace the mutated dystrophin with normal protein, several factors – including the large size of the protein – have led researchers to look for other therapeutic strategies.

About 15 percent of boys with DMD have a genetic mutation called a "premature stop codon" that instructs the protein-making machinery of the cell to stop building dystrophin before the protein is complete. In the late 1970s, scientists found that in yeast, certain



Lee Sweeney confers with Carl Morris, a postdoctoral fellow in physiology.

beefier than the average. How do you get the gene into the mouse? You smuggle it in, on a virus."

Sweeney explained that the virus "goes inside the cell looking like a normal virus, but, lo and behold, when it opens up, it's got a new gene in there that wouldn't have normally been there. So you can view it as a Trojan horse, if you like."

Narrator: "The mouse gets mighty – and stays that way."

In fact, Sweeney continued, "Their muscles never get weaker when they get old. They are just as strong when they're the equivalent of an 80- or 90-year old human as when they are the equivalent of a 20-year-old."

The reason is insulin-like growth

factor-I, injected into the specific muscle. The muscle, in the words of the narrator, then grows "at superhuman rate." Pulling back a moment from the world of superheroes, the narrator noted that the first use the IGF-I treatment is likely to be in treating muscular dystrophy.

But Sweeney reported that some people have considered different uses of the treatment: "Through e-mail, I've been approached by a lot of people, mostly weight-lifters, but even once a coach for a high-school football team." The coach asked Sweeney to inject his whole team "because he thought they could benefit from being a bit stronger." ■

— John Shea

antibiotics called aminoglycosides allow the cell machinery to ignore premature stop codons and continue to build proteins. More recently, scientists showed that this approach could be used to trick cells with similar mutations in the gene that causes cystic fibrosis to read through the mutation and produce the full-length protein that is absent in people with the disease. Sweeney reasoned that a similar approach might work in muscular dystrophy. Studies conducted in his lab demonstrated – first in cultured cells, then in a mouse model of muscular dystrophy – that the drug did induce the production of full-length dystrophin. More important, the muscles of mice treated with gentamicin resisted damage.

While potentially useful, aminoglycosides can also cause hearing loss and kidney damage when used in high doses. Because of this danger, Sweeney is working with a small biotechnology company to try to develop new drugs that have some of the same properties, but without the toxic side effects. He says he expects to be able to initiate clinical trials of these drugs within the next year.

Meanwhile, Sweeney is also pursuing his interest in gene therapy with an approach that could help people with another form of muscular dystrophy, called limb girdle muscular dystrophy (LGMD). The disease is caused by one of about a dozen genetic mutations. Both males and females can be affected by the illness, which leads to a weakening of voluntary muscles, primarily in the shoulder and hip “girdles.” Unlike Duchenne muscular dystrophy, LGMD is usually not fatal, and its symptoms range from mild to severe disability.

Some people with LGMD have mutations in genes called sarcoglycans. These are proteins in the membranes of muscle cells. When they are defective, the muscle cells are not able to react normally to the stress of muscle contraction. Sweeney has focused on mutations in one particular type of sarcoglycan, called gamma-sarcoglycan. His laboratory is working with gene-therapy colleagues at Penn

to develop vectors that will deliver normal gamma-sarcoglycan to the muscles of people with this mutation. The vector they are using is called adeno-associated virus (AAV). AAV has shown particular promise in gene therapy trials because, unlike some other potential viral vectors, AAV does not in itself cause human disease, nor does it provoke an immune response from humans.

The researchers have shown that injecting the vector carrying the normal gamma-sarcoglycan gene into the limbs of mice lacking the gene prevents degeneration of the muscles. They have also engineered the vector so it expresses gamma-sarcoglycan only in muscle cells, rather than in all types of cells. As a result, the gene therapy does not provoke an immune response.

Sweeney says he hopes that human trials of this approach can start soon. As he puts it, “This is our entry point using AAV as a vector. If it goes well, we’ll probably try to do something with Duchenne muscular dystrophy and AAV as well.” One possible approach would be to put into the vector a factor that would stimulate the production of eutrophin, a protein related to dystrophin that may be able to compensate for its loss. “We’re trying to find things that will be more generic for all of the Duchenne patients and, for that matter, for Becker patients as well.” Becker muscular dystrophy is a less common and much milder form of MD that is also caused by a defect in the dystrophin gene.

Rescuing aging muscles

Sweeney’s interest in muscle physiology intersects with his own life in other ways beyond his work with parent and advocacy groups. Watching his grandmother grow older, in fact, prompted him to ask questions about what could be done to rescue the waste of muscle that occurs naturally as people age. “My grandmother had total use of her mental faculties, but became wheelchair bound just because of progressive muscle weakness that no amount of her trying to exercise was able to overcome,” he recalls.



Elisabeth Barton, Ph.D., carried out critical gene-therapy experiments that showed increased muscle mass and strength in mice.



In fact, all mammals lose up to a third of their muscle mass and power as they age. A few years after his grandmother died, Sweeney says, he started thinking about how to approach the problem of muscle wasting, as well as the potential for gene therapy as a means to deliver factors that might reverse it. The question was, what sort of gene delivery might be useful in an aging setting?

Insulin-like growth factor I (IGF-I) is a substance that stimulates growth and repair of the muscles. Produced both by muscles themselves and by the liver, IGF-I drives protein synthesis and suppresses protein degradation. Perhaps more important, it stimulates cells called “satellite cells” in the muscle to divide, differentiate, and regenerate muscle. Researchers at other institutions had shown that injection of IGF-I into the damaged muscles of mice improved both the structure and function of those muscles.

Sweeney thought gene therapy might be a better way of delivering the factor. Elisabeth Barton, a post-doctoral fellow in Sweeney’s lab, carried out the critical experiments along with colleagues at Massachusetts General Hospital. Using the AAV virus as a vector, Barton injected the IGF-I gene into one leg of mice ranging from 2 to 24 months of age. The ages of the mice chosen represented the equivalent of adolescent, 55-year-old, and 70-year-old humans. The other leg of each mouse was left untouched as a control. (Barton is now assistant professor of anatomy and cell biology in Penn’s School of Dental Medicine.)

All of the mice showed increases in muscle mass and, most important, the injections completely prevented the age-related loss of muscle mass and significantly boosted muscle strength in the oldest mice. Sweeney presented the results of this research at the annual meeting of the American Society for Cell Biology in December 1998, which coincided with the publication of the research in the *Proceedings of the National Academy of Science*. It did not take long for news about these “mighty mice” to leak out to the popular press around

the world – and for people involved with groups like the International Olympic Committee and World Anti-Doping Agency to voice their concerns.

When his lab group first started this line of research, Sweeney says, “I didn’t really think about the athletic implications. But when we started analyzing the young mice that we were doing it in, it became pretty clear that they were getting strong without doing anything. So then, overnight, it became clear that there was going to be some interest in this from an athletic standpoint.”

“Some interest” is an understatement. Sweeney’s work has been featured in publications ranging from *Science News* to *The New York Times* to *Sports Illustrated* and broadcast on BBC News and CNN. In fact, in 2002, CNN ran what it calls an on-line “quickvote” on Sweeney’s research: “Do you think something like IGF-I – if it’s found to work in humans as it does in mice – should be applied to athletic performance?” The CNN web site tallied 6,522 votes. Twenty-eight percent voted “Yes, absolutely”; 30 percent voted “Under certain circumstances – like pure fitness – but not for competition”; and 42 percent voted “No way.” The *Weekend Australian* began an article in 2002 in dramatic fashion: “Lee Sweeney guards a genetic fountain of youth. In his Pennsylvania laboratory, the elite biotechnologist is testing a muscle-building gene designed to fend off frailty in the elderly.” In addition, Sweeney has been asked to speak about his work at meetings of the World Anti-Doping Agency, The National Human Genome Research Institute, and the President’s Council on Bioethics, among others.

“The availability of this sort of technology to an athlete in this country is not going to happen any time soon,” said Sweeney in his address before the President’s Council on Bioethics. “But on the world stage, in a world where countries in the past have shown that they want their athletes to win no matter what, and they will give them any experimental drug that might be performance enhancing no matter what the long-term

consequences, one can imagine that with enough money you could put together a program to genetically engineer your athletes and do it in such a way that it would be totally undetectable unless you were to remove tissue from that athlete. There would be nothing in the blood, no signature in the blood or urine to indicate that the tissues had been genetically manipulated.”

Nonetheless, Leon Kass, M.D., Ph.D., chairman of the President’s Council, reminded the council that the ethical issues are significant. According to Kass, an ethicist from the University of Chicago, “What’s in a way at stake in this is something like the view of the life cycle and, forgive me, a place of decline in the overall shape of a life. While nobody from a medical point of view or even from an experiential point of view would choose debility given the opportunity to avoid it, one at least has to wonder what the world would be like if you’ve got 75-year-old men quite happily playing ice hockey; and what the view of the life cycle would be if in a way what you are really aiming for – never mind the immortality research – but you’re going to get everybody up to the brick wall sort of looking and acting as if they were 30.”

The controversy surrounding the idea of genetic enhancement has obscured some of the other implications of Sweeney’s IGF-I research. In addition to helping the aged, IGF-I treatment shows promise in helping people with muscular dystrophy. Sweeney and colleagues have genetically engineered the mdx mouse (mice with the mouse version of muscular dystrophy) to churn out high levels of IGF-I as well. Sweeney reports that these mice show increases in the size and strength of their muscles, better regeneration, less muscle wasting, and less buildup of scar tissue.

Despite the publicity and controversy, Sweeney says that ethical concerns will not stop his research. “I think it’s unethical *not* to try to do something to help a population that needs medical assistance just because there might be fallout in sports. I have no ethical dilemma. It’s up to the agencies to do what they can; but it can’t stop me or

other people from trying to develop treatments that will benefit people.”

Racing towards the future

In addition to the basic research about myosin, the anticipated clinical trials studying the use of aminoglycosides in people with Duchenne muscular dystrophy, and the gene therapy trial in people with limb girdle muscular dystrophy, Sweeney has been working for several years with Tim Gardner, a Penn cardiac surgeon, to develop gene therapy approaches to treating heart dis-



Sweeney Among the Ethicists

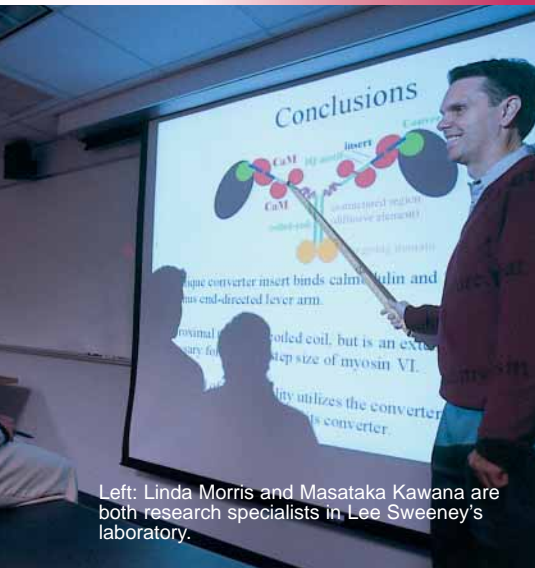
Some biomedical pioneers would hesitate to go anywhere near Penn’s Center for Bioethics. Yet last spring, H. Lee Sweeney, Ph.D., ventured to the very center of the Center to make a presentation to graduate students and other interested listeners. Among them was Arthur L. Caplan, Ph.D., director of the center and one of the most quoted bioethicists in the nation. Sweeney, chair of Penn’s Department of Physiology, was there to talk about what he called “the growing concern in the use of gene technology” when it is used not to target a specific disease or a defective gene but for “genetic enhancement.” The irony is that Sweeney’s own basic research has helped raise some of those concerns.

In the laboratory, Sweeney has introduced insulin-like growth factor I (IGF-I) into the muscles of mice by way of a viral vector. Not only did the treatment basically halt many of the effects of aging in mice, it increased their strength by some 30 percent and more. Sweeney’s hypothesis when he began this research was that the treatment might lead to better maintenance; might increase muscle regeneration after injury; and, in the young, might increase the rate of growth. One of the ethical issues Sweeney himself raised at the Center for Bioethics is

that the gene therapy has to be done *before* the loss occurs – otherwise, the loss cannot be recovered. In muscular dystrophy, Sweeney noted, “the block on fibrosis is indirect”: the IGF-I does not prevent the muscle degeneration but increases the ability of muscle to develop. In Sweeney’s view, there is less of an ethical issue when IGF-I is given to help counter muscular dystrophy. But, he continued, what about its use in young animals – and young humans looking to be stronger and faster?

According to Sweeney, the United States Olympic Committee is upset because the use of IGF-I does not change blood levels and cannot be detected without a muscle biopsy. “Athletes, I think, are always willing to take these chances,” Sweeney told the group. But, he added, this approach is more available to governments than individuals. He cited the example of East Germany, which subjected its Olympic athletes to steroids. Such treatment could provide “a little bit of a competitive edge” – but that might be all an athlete needs to win. What Sweeney called the larger ethical issue is: if the treatment is safe, why not introduce it as early as possible? Won’t it be something that everyone, some day, will want?

Noting that the technology itself



Left: Linda Morris and Masataka Kawana are both research specialists in Lee Sweeney's laboratory.

ease. They have begun to identify genes that produce factors that can rescue and stabilize hearts after a major heart attack.

If there's a downside to Sweeney's relentless pursuit of, well, everything, it's that there is not enough time in a day to accomplish all he's set out to do. "I like the advocacy groups and I like the research," he says. "You just run out of time is the problem."

Since he was named chairman of the Department of Physiology in 1999, the demands on his time have

only increased. But it's the demands from outside the University that are really taking their toll. During a recent one-month period, says Sweeney, he was only in town for three days. First, he was in Japan to give a keynote address at one scientific meeting. Because it was an exciting time for a project with collaborators in France, he went from Japan to France; and then to Berlin to speak at another meeting. Then it was back to Washington, D.C., to review grants for the NIH, and then back to Japan to give another talk at a meeting.

He has cut out, for now, his training of graduate students, feeling that he does not have the time to serve as a mentor properly. "I have to make appointments with his secretary now," says Amber Wells ruefully. She was the last graduate student to complete her doctorate in his lab. But it was not always that way, she notes. "He's brilliant and fun to talk to and bounce ideas off," she says. "He's a cerebral kind of guy."

With so much on his plate, Sweeney says he finds little or no time to enjoy his beloved opera or devote sufficient time to his other passion, wine. Nevertheless, says Wells, he is a true wine connoisseur. "It's amazing what the man can keep in his brain," she says. "He remembers every vintage he's tasted, and where and when he had it. It's like he has an amazing database in his brain."

Sweeney says he does not think that splitting his time between basic science, applied science, and public advocacy has detracted from the work he does. "I think the travel does hurt my research to some extent, but it has its plusses too." For one thing, the public exposure brings in additional money from private donations. And his stature in the science community does not seem to have suffered.

"His basic, fundamental research is top end," asserts Yale Goldman. While many scientists might find tackling both basic and applied research too demanding, continues Goldman, Sweeney seems to thrive. "It's impressive that he is making an impact in both of these areas. He covers a broad range, and he does it very well." ■

does not appear to be hard, Caplan asked about the cost involved. "Not trivial," said Sweeney, estimating that to treat an athlete's two legs might cost \$100,000.

Another person wondered about the risk of cancer in the IGF-I treatment. Sweeney said it was not common; he has not seen it in mice that have two-to-three times the normal levels of IGF-I.

The guardians of the Olympics, suggested Caplan, seem primarily concerned about the distortion of body endowments and issues of fairness and "naturalness." He wondered whether the current vogue of "extreme sports" might lead people to try genetic enhancement—for example, if a producer said, "I'd like to see someone carry four cars up a ladder." But "where the pressure may come more," he added, was in the matter of aging, especially if wealthy individuals are involved. Said Sweeney, "Frankly, I'm a little surprised it hasn't happened."

Sweeney also briefly described his meeting with the President's Council on Bioethics. His sense was that the council on the whole was opposed to genetic manipulation of the sort that IGF-I treatments would make possible. It would force us to redefine "what we know as human," whereas those on the council view aging "as a normal

part of life." Yet Sweeney did not seem persuaded. Shouldn't it be a matter of individual choice? No one *has* to undergo the IGF-I treatment.

On the other hand, suggested one of the bioethics students, if you refuse the treatment, are you in effect making yourself a burden on society as you age?

Would the treatment lengthen life? Again, Sweeney reported that his research group has not seen evidence among the animal models. If there was any difference in life spans, he speculated, it would be a trivial amount.

Caplan argued that our notion of "what's normal aging is a modern construction." In fact, for much of humanity's past, people did not live much beyond 40 or 50. Yet he said he could understand how the prospect of seeing elderly people in the future blithely rope-climbing could "freak people out."

The session closed as Caplan asked how many scientists were working in this area so far. "Probably just half a dozen," replied Sweeney. In clinical trials, dogs will be next, and he estimated that human trials are about four years away. In the meantime, joked Caplan, referring to the Center for Bioethics and the budding ethicists in the room, "you're generating business for us." ■

— John Shea