MUSCULAR AGAIN

Within a decade or two, scientists will create a genetic vaccine that increases muscle mass—without exercise. **By Glenn Zorpette**

IF PHYSICAL BEAUTY is an evanescent mélange, its foundation—a set of firm, shapely muscles—is just as fleeting. Bright, limpid eyes, good facial structure and lustrous hair can all persist, but muscles eventually wane.

Regular exercise can slow the decline quite a bit, and the rate of loss varies from person to person. Still, if you manage to be a healthy septuagenarian, chances are you will have lost at least 20 percent—and perhaps as much as one third—of the muscle mass you had in your late 20s.

Unfortunately for you, far more than your animal magnetism is at stake as your precious thews wither like a praying mantis in a pottery kiln. Muscle is the most abundant tissue in your body, which makes it the largest store of a variety of key substances. These include amino acids, the building blocks of the proteins that make up the struts and girders of cells and, in the form of enzymes, carry out the biochemical processes of life. For this reason, among others, the loss of muscle can actually weaken your immune system. Geriatric health specialists now also see muscle loss as underlying many of the injuries to elderly people caused by falling. Thrown off balance, an older person may not have the muscle power necessary to correct posture quickly enough to avoid a nasty fall.

With relatively few old-timers showing an inclination to pump iron three times a week for the rest of their lives, the potential market for an alternative muscle-building drug is clearly enormous. And science finally appears close to creating one. In separate experiments over the past couple of years at the University of Pennsylvania Medical Center in Philadelphia and at the Royal Free and University College Medical School in London, researchers tested muscle-building vaccines based on engineered genes. Injected into mice, the vaccines boosted muscle mass in the animals' legs by 15 to 27 percent. Amazingly, the increases were measurable in only a month or so and didn't require any exercise at all.

Lest couch potatoes rejoice, several major obstacles would have to be overcome before injections let inactive senior citizens go from park benches to bench presses. Still, many muscle researchers believe that human tests are inevitable, and some think the first ones will occur within the next couple of years. Not only would such a vaccine be about as close as humanity is likely to come anytime soon to an antiaging elixir, but it could also be a major breakthrough for the treatment of a host of degenerative muscle diseases, including the various forms of muscular dystrophy.

Genetic muscle-building **vaccines** would be **essentially undetectable** in the body.

On the downside, it takes little imagination to see the possibilities for abuse of the vaccines by healthy young athletes in power sports like football, sprinting and short-distance swimming. Compared with anabolic steroids, the modern-day illegal but ubiquitous muscle-building drug of choice, a vaccine based on an engineered gene would offer some major advantages. It would need to be administered only one time, rather than periodically, and it would be essentially undetectable in the body.

The mere likelihood of a muscle-building drug with those features is already causing anxiety in international sports circles. "When you come to a method where you are increasing proteins in the cells genetically and directly, you'll have to have much more sophisticated detection techniques," says Mats Garle, scientific director of the Doping Control Laboratory of Huddinge University

Hospital in Sweden. The laboratory, which tests elite athletes, is one of the best of its kind in the world. "Maybe we'll never get a solution to that problem," Garle concedes.

MUSCLES 101

Muscle is among the strangest tissues in the human body. A single muscle cell consists of a membrane, many scattered nuclei that contain genes, and thousands of inner strands called myofibrils that constitute the cytoplasm of the cell. Sustained by the multiple nuclei, the cells can grow to be centimeters long.

Filling the inside of a muscle fiber, the myofibrils can be as long as the fiber and are the part that enables the cell to contract forcefully in response to nerve impulses. The actual contraction is accomplished by the myofibrils' tiny component units, which are called sarcomeres. They are linked end to end to make up a myofibril, which contracts when all of its sarcomeres do. Within each sarcomere are two filamentary proteins, called myosin and actin, whose interaction causes contraction. Basically, during contraction, a sarcomere shortens like a collapsing telescope, as the actin filaments at each end of a central myosin filament slide toward the myosin's center [see "The Mystery of Muscle," by Glenn Zorpette; SCIENTIFIC AMERICAN PRESENTS: Men: The Scientific Truth about Their Work, Play, Health and Passions, Summer 1999].

Muscle cells, also known as fibers, cannot split themselves to form completely new fibers. So a muscle can become more massive only when its individual fibers become thicker.

What causes this thickening is the creation of new myofibrils. In an extremely complex process that is still poorly understood, the mechanical stresses that exercise exerts on tendons and other structures connected to the muscle trigger many different biochemical pathways that ultimately cause the muscle cells to make more proteins. Enormous amounts of these proteins, chiefly myosin and actin, are needed as the cell produces additional myofibrils.

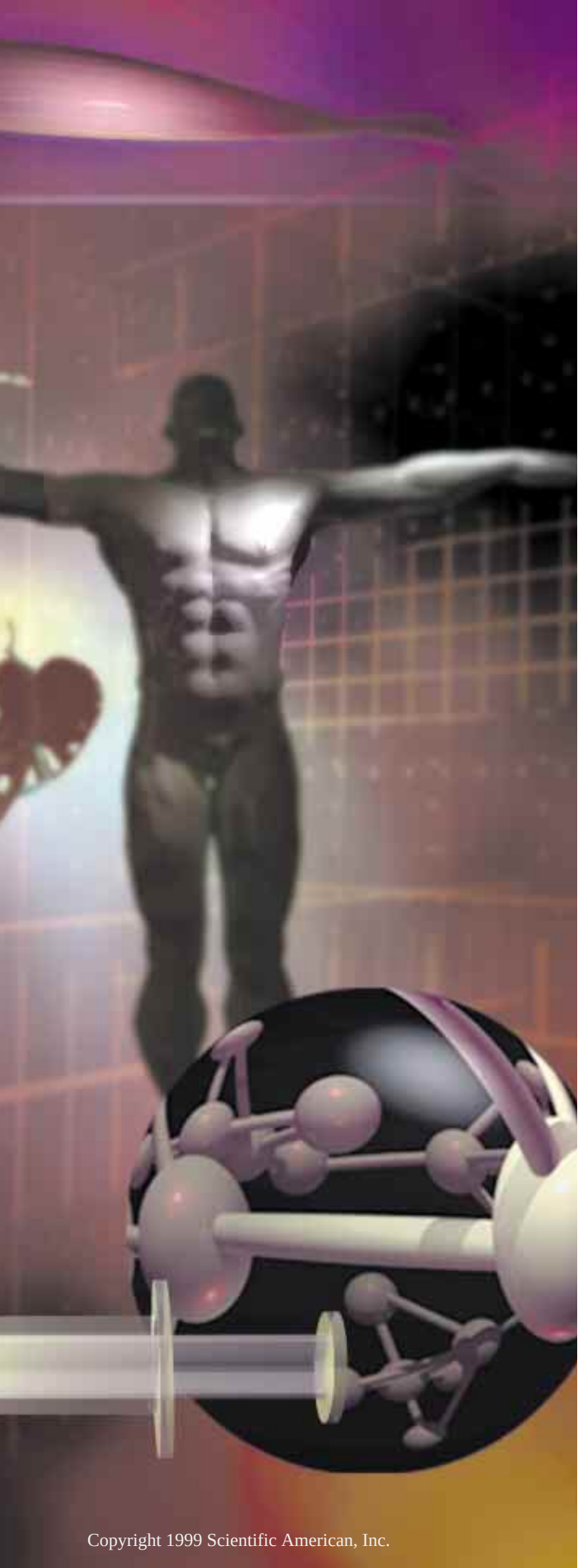
To produce and support all this protein requires more nuclei. As muscle cells cannot divide, the new nuclei are donated by so-called satellite cells, which are scattered among the many nuclei on the surface of a skeletal muscle fiber. Satellite cells are largely separate from the muscle cell and, unlike it, have only the usual one nucleus apiece. Thus, they can replicate by dividing.

Researchers now know that satellite cells proliferate in response to the stresses and wear and tear of exercise. As they multiply, some remain as satellites on the fiber, but others become incorporated into it. Their nuclei become indistinguishable from the muscle cell's other nuclei. With these additional nuclei, the fiber is able to churn out more proteins and create more myofibrils.

According to the prevailing theory, rigorous exercise inflicts tiny "microtears" in muscle fibers. The damaged area attracts the satellite cells, which incorporate themselves into the muscle tissue and begin producing proteins to fill the gap. Significantly, the number of nuclei passing from the satellite cells into the damaged area of the fiber is greater than the number of nuclei lost when the gap opened up. As a result, in that part of the fiber, more protein can be produced and supported. Gradually, as more microtears are repaired in this

Genetic vaccines now being developed to help the elderly increase their muscle mass will inevitably be abused by athletes and bodybuilders.

ALL ILLUSTRATIONS BY ALFRED T. KAMAJIAN



manner, the overall number of nuclei grows, as does the fiber itself.

In order to produce a protein, a muscle cell—like any cell in the body—must have a "blueprint" to specify the order in which amino acids should be put together to make the protein—that is, which protein will be created. This blueprint is a gene in the cell's nucleus, and the process by which the information gets out of the nucleus into the cytoplasm, where the protein will be made, starts with transcription. It occurs in the nucleus when a gene's information (encoded in DNA) is copied into a molecule called messenger RNA. The mRNA then carries this information outside the nucleus to structures known as ribosomes, which assemble amino acids into the protein—myosin or actin, say—specified by that gene. This latter process is called translation.

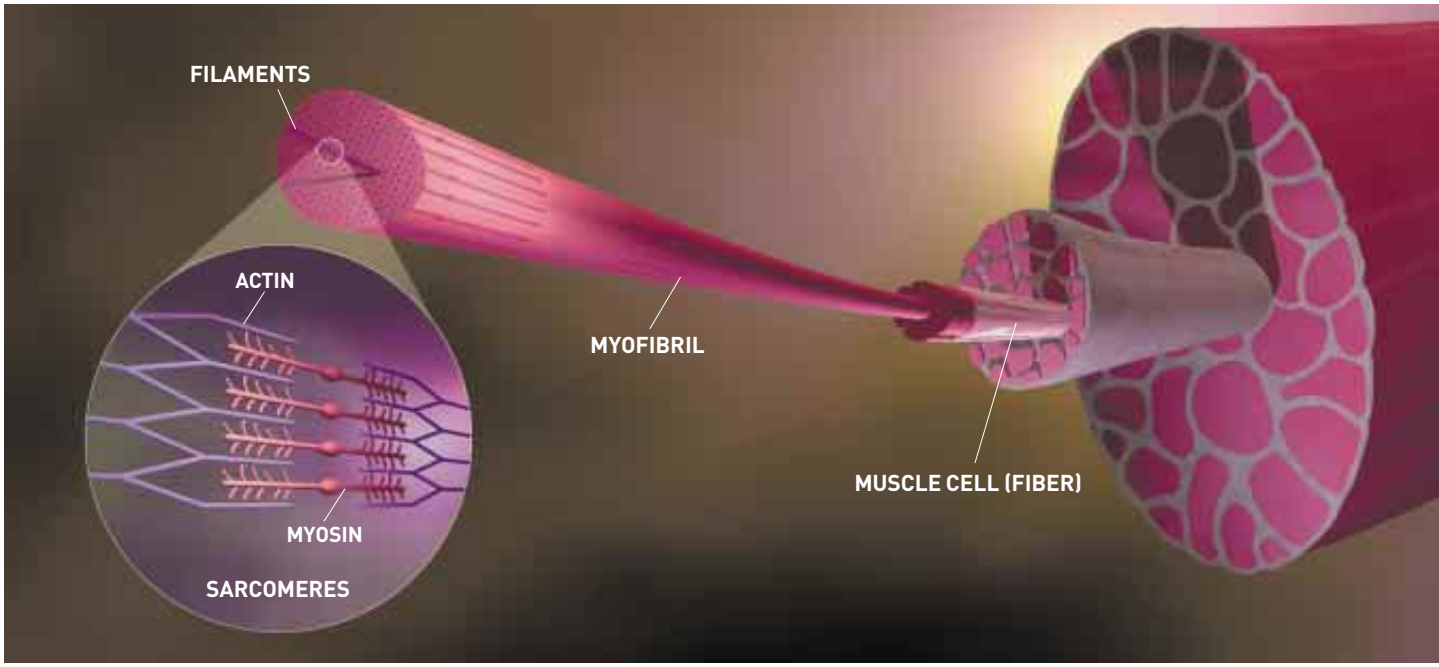
The source of biochemical complexity in muscle enlargement is not really transcription or translation but rather what precedes those processes: the many biochemical pathways that bring about transcription. Researchers know of dozens of different key biochemicals that initiate or sustain these pathways, and some suspect that there may actually be thousands. Most of these biochemicals are proteins that fall into five basic categories: sex hormones, like testosterone; thyroid hormones; insulinlike growth factors; fibroblast growth factor; and myriad other proteins lumped under the general term transcription factors. Some of these proteins are produced in organs such as the liver and circulate throughout the body; others are created locally, in specific muscle tissue, in response to exercise or stretching of that tissue.

These hormones, growth factors and transcription factors act in a variety of ways, often in conjunction with one another, to promote protein production. The many biochemical reactions are like a sprawling game with thousands of players, the goal being to get into the nucleus and, typically, to combine with a site on a chromosome known as a promoter region. This combination activates a nearby gene and triggers transcription.

As with any game, there are rules. Only the transcription factors, as their name implies, can get into the nucleus by themselves and activate genes. Hormones and growth factors spur transcription indirectly, usually in conjunction with transcription factors and other molecules called receptors. And one of the game's complexities is that sometimes transcription factors activate genes that produce more transcription factors.

As an example of how a hormone works, take testosterone. Produced by the testes and carried by the blood, it can penetrate a muscle cell's outer membrane and get into the cytoplasm. There it combines with a receptor floating free in the cytoplasm. The complex then enters the nucleus and binds to a promoter region to activate a gene and initiate transcription. Because anabolic steroids are merely synthetic versions of testosterone, this pathway is the one they trigger and exploit to build muscle.

Other pathways are even more complex. Some crucial ones begin with the binding of growth factors, for instance, to receptors that poke through the surface membranes of cells. When the parts outside the cell bind to a specific molecule, the union activates a series of chemical reactions inside the cell. For example, the binding of a growth factor to its receptor activates cascades of enzymes, called kinases, that modify other proteins in the cytoplasm, which in turn bind to promoter regions on chromosomes and otherwise regulate the activity of genes.



One of the most important growth factors is insulinlike growth factor-1 (IGF-1). During infancy and childhood, IGF-1 produced by the liver circulates throughout the body, rapidly expanding all the body's muscle fibers. The amount of this circulating, liver-produced IGF-1 eventually declines sharply, ending the early-life growth spurt. For muscle growth, the free ride is then over, and only exercise can add (and eventually, merely maintain) muscle mass. IGF-1 and other growth factors continue to play a major role, but they are released only locally in muscle during exercise or in response to injury.

Significantly, IGF-1 concentrations are high around the tiny tears in muscle fibers caused by exercise. Researchers believe that the growth factor plays a major role in attracting the satellite cells to the damaged area.

MIGHTY MICE

It was this local, muscle-specific form of IGF-1 that the University of Pennsylvania researchers exploited in their genetic experiments on mice. The Penn team, led by H. Lee Sweeney, took the gene that codes for the rodent form of muscle-specific IGF-1 and put it in a virus. Viruses can be useful for splicing engineered genes into cells because they target the nucleus, inserting the genes into a chromosome so that the DNA is not lost over time.

Besides the gene for IGF-1, the virus's DNA payload consists of other genetic material, such as a promoter region. Sweeney and his colleagues designed the promoter region so that it would always be "on," in effect inducing transcription of the *IGF-1* gene all the time.

Injected into a mouse's leg, the virus eventually got into 50 to 75 percent of the leg's muscle cells, Sweeney estimates. In each cell, the virus entered the cytoplasm and broke up, releasing the engineered gene and the associated genetic material. By mechanisms not well understood, the gene and other DNA became integrated into the nucleus's own DNA. Intriguingly, the invading DNA seemed to position itself randomly on a chromosome, Sweeney reports.

Once on the chromosome, with its promoter region stuck in the on position, the engineered gene started transcribing mRNA for muscle-specific IGF-1. The transcription continued until the animal died, of old age.

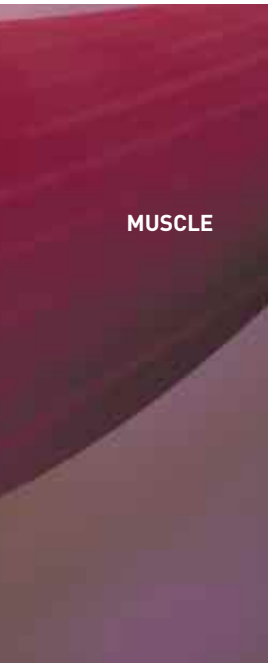
Sweeney has so far performed dozens of trials on both young and older mice. When injected at a young age, the mice grew to be adults with 15 to 20 percent more muscle mass in the treated area than they would have had otherwise. Injected at maturity, on the other hand, the animals did not gain muscle mass but—significantly—they did not lose much, either. By keeping what they had, the elderly mice had on average 27 percent more muscle mass than their untreated counterparts. In mid-June, Sweeney was exercising four dozen treated animals to see whether exercise added any effects to treatment.

The University College London experiments were broadly similar to those at Penn. In the British experiments, the muscle-enhancing substance the cells were tricked into producing was one that the lead researcher, Geoffrey Goldspink, dubbed mechano-growth factor (MGF). The genes for mechano-growth factor and IGF-1 are so similar that mechano-growth factor is considered a form (the technical term is "isoform") of IGF-1.

As a vehicle to deliver the engineered gene to the muscle cells, however, the British researchers chose not a virus but a plasmid, a circular piece of DNA. Although arguably safer than viruses, plasmids are much less efficient at getting into cells. Also, a plasmid does not insert itself into a chromosome, so it eventually stops prompting production of the protein.

Nevertheless, some of the injected plasmids do get into nuclei (again, through mechanisms not well understood). The plasmids,

Injected at a **young age**, the mice **grew** to have **15 to 20 percent more muscle.**



Muscle consists of cells full of strands called myofibrils, which are in turn made up of contractile units called sarcomeres. The key components of a sarcomere (*inset, exploded view*) are myosin (*red*) and actin (*lavender*). These protein molecules slide over one another as the sarcomere contracts and uncontracts. The myosin heads, which protrude outward from the filament's central stem, lock onto sites on the closest actin. The heads release one site and grip the next, "walking" the actin over the myosin.

too, had been given promoter regions stuck in the on position. So as soon as the plasmids got into muscle cell nuclei, they started cranking out mRNA for mechano-growth factor. Higher levels of MGF followed, and then an increase in muscle. According to Goldspink, injections into the legs of mice produced muscle mass increases of up to 20 percent—without exercise.

Weight lifters contemplating canceling their health-club memberships should read on. As it stands today, once a gene for a growth factor gets into a chromosome in a muscle cell nucleus, the cell continues churning out elevated quantities of the growth factor for as long as the animal lives.

"In principle the only thing lacking is a control mechanism to keep a hold on it," says Peter Schjerling, a research geneticist at the Copenhagen Muscle Research Center, which is affiliated with the University of Copenhagen and the city's University Hospital. "And there are a lot of people working on that," Schjerling adds.

The leading contender is known as a regulated promoter. It would be used in conjunction with a drug that turns the promoter region on and off. So long as the patient takes the drug, the promoter region of the engineered gene that has ensconced itself into the nucleus of some of his or her cells will be on, and the cells will produce the specified protein. When the patient stops taking the drug, the promoter region will switch off.

The problem is that many of the drugs for the experimental regulated promoters now available have toxic side effects. Still, Penn's Sweeney believes improvements are inevitable. "Better regulated promoters will come along," he asserts. "They are of too much interest to the biotechnology community. There are a lot of pharmaceutical companies working on them right now."

So far there have been no experiments with human subjects using the specific kind of virus (an adeno-associated virus) that Sweeney and colleagues are using. This situation is likely to change in the near future, however. A team led by James M. Wilson at the University of Pennsylvania has already used such a technique to produce in monkeys a protein called alpha sarcoglycan. Lack of the protein is a major factor in a type of muscular dystrophy that selectively affects the arms, legs and hips. The experiments with the monkeys have gone well, and within a year or two Wilson's team expects to begin trials of the treatment with humans afflicted with the disease, which is termed limb-girdle muscular dystrophy.

If that trial succeeds, Sweeney hopes to begin tests of his own, to produce muscle-specific IGF-1 in patients whose muscular dystrophy is so severe that they have no other recourse. If those trials go well, he will write a proposal to expand the experiments to include elderly people whose only health complaint is age-related muscle loss.

Not long after that, black-market versions of the genetic vaccines will inevitably begin flowing into the demimonde of bodybuilders, professional sports stars and international athletes, for whom even small increases in muscle can mean millions of dollars, greater prestige or both. "If in 20 years these viruses are available to treat muscular disease, they will be available to athletes seeking to gain a competitive edge," Sweeney notes. But, he continues, "it's not going to stop us from developing treatments for degenerative diseases."

GETTING PUMPED THE EASY WAY

It is easy to see how the narcissistic would find the drug irresistible. It would build muscle mainly where it was injected, making it possible for even the lazy and uncoordinated to sculpt their bodies by doing nothing more strenuous than lifting a hypodermic needle. Big biceps, nice calves and bulging pectorals will all be just a few injections away.

Of course, an instant physique of this kind will not come without a physiological price. To improve performance or look really buff, athletes and bodybuilders will probably need to take considerably larger doses than what doctors will prescribe for therapy. Thus, they would probably suffer some of the known or suspected side effects of abuse of IGF-1, such as an enlarged heart and, possibly, cardiac arrest. As with anabolic steroids, abuse of the genetic vaccine will most likely turn out to be more Faustian bargain than free ride.

Because the engineered genes would be copies of those that are normally in the nuclei of muscle cells, sports officials would find it extremely difficult to detect the abuse of a genetic vaccine—even if they were allowed to take a muscle biopsy. Today, however, biopsies are not permitted as part of a routine antidoping test. "Nor do I think athletes would be happy about submitting to a muscle biopsy just before a competitive event," Sweeney remarks.

From Samson's hair to Popeye's spinach, the idea of a strength-boosting talisman has long captivated us. Now, as science is poised to produce one, we might think about it in light of the varied motives that invariably accompany the application of any powerful new technology. It is hard to overstate the value of a genetic treatment that could let millions, perhaps billions, of people be more active, independent and resilient, improving their quality of life immeasurably. But it is anybody's guess what athletic competition will be like in an age of undetectable genetic enhancements.

"It could be like bodybuilding is today, where if you want to compete at the top level, you have no choice but to take anabolic steroids," says Garle, the antidoping expert.

Håkan Nyberg of the Swedish Sports Confederation is more optimistic. If in 15 or 20 years an age of genetically doped international superathletes arrives, he asks, "will sport keep its market value? I'm not sure. The driving force today is people like us who watch the competitions. Will we like watching a circus of artificial animals?"

ABOUT THE AUTHOR

GLENN ZORPETTE is co-editor of this issue of SCIENTIFIC AMERICAN PRESENTS. He donated muscle tissue from his thigh for use in some of the research described in this article.