SCIENCE NEWS of the week

3-D Atomic View of Muscle Molecule

Scientists have known for decades that two proteins called actin and myosin interact to make muscles contract. In muscle cells, these proteins bundle into filaments, with myosin overlying actin and pulling itself along actin to shorten muscle fibers. Myosin obtains the chemical energy needed to fuel this shortening by breaking phosphate off adenosine triphosphate (ATP) molecules.

Now, researchers can take an in-depth look at how this molecular motor transforms chemical energy into motion, says Ivan Rayment, a crystallographer at the University of Wisconsin-Madison. In the July 2 SCIENCE, he, Wisconsin colleague Hazel M. Holden, and their collaborators present a detailed, three-dimensional picture of myosin. They then combine their findings with earlier results from the Scripps Research Institute in La Jolla, Calif., and from the Max Planck Institute for Medical Research in Heidelberg, Germany. "What this work does is tie [previous results] together," says Rayment.

The synthesis confirms current ideas about actin and myosin and fills in some missing details, comments Edwin W. Taylor of the University of Chicago.

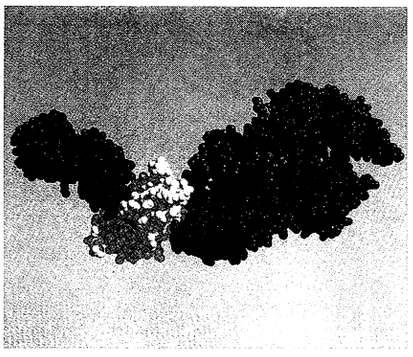
"Now you have the 3-D structures of the two major players [actin and myosin]," adds Ralph G. Yount, a protein biochemist at Washington State University in Pullman. "You can begin to figure out how they work on a molecular basis."

Until now, the actual structure of the myosin molecule had eluded scientists, Rayment says. Try as they might, they could not grow crystals of this very soluble protein, a necessary first step for doing X-ray diffraction studies to pinpoint the location of the atoms in the myosin molecule.

Then, a decade ago, Rayment modified dissolved myosin by adding methyl side groups to some of the amino acids that make up the protein, thus obtaining crystals. He and his colleagues spent the next six years working out a way to make each myosin molecule in the solution take up the same number of methyl side groups in the same places to ensure that a pure crystal formed.

Myosin consists of two interwoven protein fragments, or "heavy chains." Each fragment has a fat "head," with two smaller peptide chains attached, and a tail. Rayment's group made crystals of single head fragments.

The new data confirm that one side of myosin's head contains a binding site for ATP. Actin attaches on the opposite side of the head. The structure also shows that the head's two peptide "light chains," each about 150 amino acids long, cling tightly to the head. Unexpectedly, however, the amino acids in the head also fold to form a cleft along the middle.



Rayment *et al.*/Univ. Wis.

Computer graphic shows myosin fragment's light chains (yellow, magenta) wrapped tightly about its heavy chain "head," which contains an ATP binding site (green) separated by a horizontal cleft from where actin attaches at lower right corner.

"You can now see how the atoms can be interacting and what changes are taking place to [cause] tension," says Richard W. Lymn, a muscle biophysicist at the National Institute of Arthritis and Musculoskeletal and Skin Diseases in Bethesda, Md.

Rayment and his colleagues think that when ATP attaches, it causes the narrow cleft to widen. This motion splits the binding site for actin and loosens myosin's hold on actin. Then myosin bends, encircles the ATP, and chops off a phosphate. This causes yet another shift in myosin's structure so actin can reattach.

"[This shift] closes the cleft, squeezes out phosphate, and the molecule pops open," Rayment explains. The initial bending strains the molecule — like stretching a rubber band. The reclosing of the cleft releases that strain, and the rebound of about 5 nanometers causes myosin to slide over actin, creating the "power stroke" for contraction. The light chains extend the distance of this shifting in the cleft, making a longer lever, he adds.

"It's landmark research," comments Yount. "It's the sort of thing that will wind up in every biology textbook."

— E. Pennisi

Pesticides in produce may threaten kids

Many fruits and vegetables sold in the United States contain one or more pesticides. In general, these residues are low and within concentrations allowed by law. However, because the foods they taint make up such a large proportion of a young child's diet, children may be ingesting unsafe quantities of toxic agricultural chemicals. Or so conclude a pair of reports issued this week.

"If you eat, you eat pesticides," asserts Richard Wiles of the Environmental Working Group (EWG) in Washington, D.C., a new, nonprofit spinoff of the Center for Resource Economics.

For *Pesticides in Children's Food,* the report EWG issued Monday, Wiles examined previously unpublished residue data on 17,000 food samples tested at Food and Drug Administration (FDA) laboratories nationwide and 3,000 samples analyzed for supermarkets by independent labs. He then coupled these data — all for foods available between 1990 and 1992 — to federal estimates of children's consumption patterns and compared the resulting exposure estimates with health-risk data.

The analysis suggests that more than one-third of a child's lifetime exposure to and cancer risk from some pesticides will accumulate by age 5. Indeed, by his or her first birthday, the average American child's exposure to some carcinogenic pesticides will exceed the federal government's lifetime acceptable-cancer-risk threshold, calculated to result in one malignancy in every million individuals.

Though Wiles says this exposure is "completely unacceptable," he says the risk involved is small and does not warrant avoiding fruits and vegetables.

"We're not talking about a food panic here," agrees Philip J. Landrigan of Mt. Sinai School of Medicine in New York City, chairman of a National Academy of Sciences (NAS) panel that reviews related issues in another report issued this week. "Parents should continue to emphasize fruits and vegetables in their children's diets."

However, the NAS panel's investigation of federal practices to limit pesticide contamination of food indicts the regulatory status quo.

The main problem, Landrigan says, is that the government has taken a "one size fits all approach," basing pesticide-risk evaluations on the diet of a typical adult. But "children differ substantially from adults, not only in size but also in metabolism and in what they eat — and therefore in the pesticides to which they are exposed," he points out. To account for that, he says, "basic changes are needed in the current regulatory system."

To improve regulations, the NAS committee advocates that the government:

- conduct food consumption surveys of children to establish diets typical of spe-

cific age groups;

• develop toxicity tests for pesticides tailored to the unique physiology of infants and children;

• make residue limits take into account potential nondietary exposures to pesticides; and

• cut by as much as 90 percent allowable residues of pesticides that may be toxic to children or for which toxicity data remain inconclusive.

Federal researchers have also analyzed pesticide residues in the context of what children eat. In a study published earlier this month, Norma Yess and her colleagues at FDA in Washington, D.C., reviewed data from food assays by the agency's chemists between 1985 and 1991. Though their study includes data on baked goods, infant cereals, infant formulas, and combination dinners (including meat), it focuses on data from 10,600 samples of fresh apples, oranges, bananas, pears, milk, and fruit juices.

A 1992 FDA analysis found that among domestically produced foods in 1991, roughly 40 percent of grains and grain products, 51 percent of fruits, and 32 percent of vegetables contained pesticide residues, notes Ellis Gunderson, a coauthor of the new FDA report.

But pesticide concentrations tend to be within federally allowed limits. Indeed, among the six years of test data FDA analyzed for its new report, less than 0.5 percent of sampled foods violated those limits, the researchers report in the May-June JOURNAL OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS INTERNATIONAL.

Raw foods tended to have the highest residues, the FDA team found. That's not surprising, they say, because these foods are tested before being washed, peeled, or processed – factors that can reduce pesticide residues by as much as 99 percent. Basing exposure estimates on these residues would probably exaggerate the amount consumers actually eat.

In a broader sense, however, "FDA seriously underreports pesticide residues in the food supply," Wiles charges.

While FDA can screen foods for more than 300 pesticides, not all of its laboratories employ all applicable tests. Among 12 regional FDA labs, seven used three or more multiple-residue screening techniques on 80 percent or more of the foods they tested, the EWG study found. The other five used just one or two screens to test 75 percent or more of their food samples. Not surprisingly, Wiles reports, "the seven most rigorous FDA labs reported twice the percentage of samples with detectable residues of one or more pesticides in apples, pears, bananas, tomatoes, and green beans."

Although FDA's data establish that crops bear multiple residues, federal agencies regulate pesticides as if exposure occurred individually and in isolation, the NAS report notes. In fact, multiple residues on a single crop are common, the EWG study indicates.

The independent labs' analyses of thousands of produce samples from supermarket warehouses indicate that residues of two or more pesticides occur on 62 percent of oranges, 44 percent of apples, and at least 25 percent of all cherries, peaches, strawberries, celery, pears, grapes, and leaf lettuce, notes Wiles. Some carried residues of six to eight pesticides, of which two or more might be suspected carcinogens, he says.

Pending data on how these pesticides may interact, regulators should consider taking a more conservative approach "by assigning toxicity equivalence factors to each of the compounds having a common mechanism of action" and then adding them, the NAS panel argues.

NAS tested this concept with five potentially nerve-damaging organophosphate insecticides used on foods. Based on residues observed for specific crops, the NAS committee found there were at least "weak" data to suggest "that for some children, exposures could be sufficiently high to produce symptoms of acute organophosphate pesticide poisoning."

The NAS panel emphasizes that it found no data showing that any pesticide residues have actually harmed children or infants. However, it did find that certain behaviors – such as eating patterns, food-preparation techniques, and pesticide-use patterns – might combine to put some young children at risk.

Briefed on both the EWG and NAS reports before their release, the EPA, FDA, and the Agriculture Department issued a joint statement on June 25. In it, the Clinton administration pledged to intensify efforts to reduce the use of high-risk pesticides and to develop safer pesticides through regulatory reform and new incentives to pesticide manufacturers. The statement added, "We expect to use the upcoming reports of the NAS and the EWG on children and pesticides as a basis for formulating the legislative and regulatory policies needed to put the administration principles into effect."

– J. Raloff and D. Pendick

A curvy path leads to Fermat's last theorem

After more than 300 years, Fermat's last theorem may finally live up to its common designation as a theorem. In a dramatic announcement that caught the mathematical community completely by surprise, Andrew Wiles of Princeton University revealed last week that he had proved major parts of a significant conjecture in number theory. These results, in turn, establish the truth of Fermat's famous, devilishly simple conjecture.

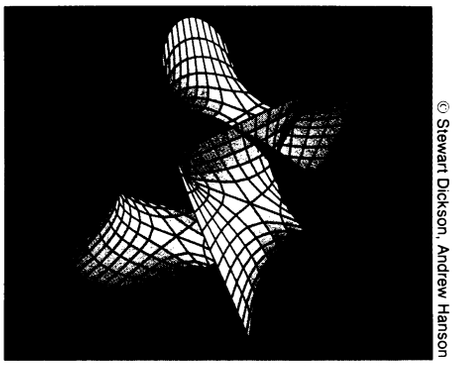
"It's an amazing piece of work," says Peter C. Sarnak, one of Wiles' Princeton colleagues. "The proof hasn't been totally checked, but it's very convincing."

Pierre de Fermat's last theorem goes back to the 17th century, when the French jurist and mathematician asserted that for any whole number n greater than 2, the equation $x^n + y^n = z^n$ has no solution for which x, y, and z are all whole numbers greater than zero.

Fermat scribbled his conjecture in the margin of a page in a mathematics book he was reading. Then, in a tantalizing sentence that was to haunt mathematicians for centuries to come, he added that although he had a wonderful proof of the theorem, he didn't have room to write it.

After Fermat died, scholars could find no trace of the proof in any of his papers. Later, mathematicians proved the conjecture for the exponent $n = 3$ and solved several other special cases. Last year, a massive computer-aided effort by J.P. Buhler of Reed College in Portland, Ore., and Richard E. Crandall of NeXT Computer Inc., in Redwood City, Calif., verified Fermat's last theorem for exponents up to 4 million.

Meanwhile, mathematicians had picked up some valuable hints of a potential avenue to a general proof that the conjecture is true. In the mid-1980s, Gerhard Frey of the University of the Saarlands in Saarbrucken, Germany, unexpectedly uncovered an intriguing link between Fermat's conjecture and a seemingly unrelated branch of mathematics. He found a way to express Fermat's last theorem as a conjecture about elliptic curves – equations generally written in the form $y^2 = x^3 + ax^2 + bx + c$, where a, b, and c are constants.



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Visualization of curves associated with the Fermat equation for n = 3.

This brought Fermat's problem into an area of mathematics for which mathematicians had already developed a wide range of techniques for solving problems. A number of mathematicians, including Barry Mazur of Harvard University and Kenneth A. Ribet of the University of California, Berkeley, followed up Frey's surprising insight with additional results that ultimately tied Fermat's last theorem to a central conjecture in number theory (SN: 6/20/87, p.397).

Named for Japanese mathematician