Prodigious protein production *sans* cells

In the biotech equivalent of leading a horse to water and trying to make it drink, gene engineers often find they can insert a new gene into living cells but still cannot get those cells to produce much useful protein. In some cases, other cell products break down the protein, or the protein might be toxic to the cells that produce it.

Now five Soviet scientists report creating a highly efficient system for making proteins without using living cells, thereby bypassing many of these sorts of problems. Their system, detailed in the Nov. 25 SCIENCE, should "open new fields" for scientific and biotechnological development, they say.

"Cell-free" systems throw together genes, amino acids, the cell's protein-making machinery and a source of chemical energy into a solution to make proteins without cell membranes to contain the reaction. The drawback common to such systems has been that they produce very little protein, say Alexander S. Spirin and his colleagues at the Institute of Protein Research in Moscow. Typically such systems produce only two to three proteins per gene copy, they say.

But the Soviet scientists have developed a "flow-through" system that creates hundreds of times as much protein as previously possible, they report. In the new system, the cell machinery and messenger RNA (mRNA) copies of the desired gene are held in a reaction chamber while a fresh solution containing the energy-storing molecules ATP and GTP continuously flows through the chamber. The flow of solution not only replenishes the supply of amino acids, ATP and GTP in the chamber, but also carries away the proteins already produced. Filters keep the larger protein-making machinery and mRNA from leaving the chamber.

Spirin and his colleagues say they kept this reaction going for more than 40 hours, producing up to 300 copies of protein per gene copy. The efficiency of the new system surprised even the researchers, who say they had expected to see some of the mRNA chewed up by enzymes that are difficult to remove from the reaction.

They also expected the efficiency of the reaction to decrease over time as some of the smaller pieces of the protein-making machinery slipped through the filters. This didn't happen either, perhaps because such molecules were always bound up with other, larger molecules, they say. The huge increase in reaction efficiency may make the system useful for creating difficult-to-manufacture proteins, several scientists note.

"You might want to make toxic proteins that can't be produced in cells," says Shing Chang, director of research at Cetus Corp. in Emeryville, Calif. Such proteins, which would quickly kill any cell that made them, could be attached to monoclonal antibodies that could then seek out and kill cancer cells, Chang says.

One drawback to the system might be the high cost of constantly replacing the expensive ATP, Chang says. "ATP is like currency," he says. "The utility of the system depends on how much you have to spend."

There are also questions of whether the process will work on the very large scale required for industrial use and whether it can produce structurally correct proteins, Chang says.

"Certain modifications of proteins occur in [the cell]," Chang says. "I would want to know if this system could make those modifications too."

The new cell-free expression system is not the best system for the production of all proteins, but it may be best for certain kinds of proteins, the Soviet scientists say. It can be used to produce not only toxic proteins but also proteins that are unstable and need to be isolated quickly, they say. It may also give researchers simple proteins to study how proteins are modified and folded by cellular machinery, and "significantly extend" the possibilities of protein engineering, they add. *— C. Vaughan*

Heart studies add to fish-oil controversy

As consumers rush to buy fish-oil capsules as a protection against coronary artery disease, scientists are mulling over equivocal results presented last week at the American Heart Association meeting in Washington, D.C.

In the past decade, studies have shown that fish-oil fatty acids seem to protect against atherosclerosis, a fatty buildup on artery walls. That evidence suggested a role for fish oil in treating coronary angioplasty patients, who are at high risk of vessel reclosure. The angioplasty procedure opens clogged blood vessels but injures them in the process. Up to 40 percent of angioplasty patients experience arterial renarrowing, which can lead to a heart attack or stroke. Drugs such as aspirin have failed to prevent vessel closure after angioplasty, but researchers had high hopes for fish oil. However, scientists studying the treatment offer mixed reviews.

Weighing in with positive evidence, Mark R. Milner of the Washington (D.C.) Hospital Center and his colleagues report that large fish-oil doses can "dramatically improve" the results of angioplasty. Milner and other scientists suggest the omega-3 fatty acids in fish oil protect angioplasty patients by minimizing vessel inflammation and scarring. Angioplasty often tears the vessel wall, possibly leading to a buildup of platelets, blood fats and scar tissue that later close the artery.

Milner's team looked at 194 angioplasty patients who were randomly assigned to treatment or control groups. Those in the fish-oil group took nine capsules containing 4.5 grams of omega-3 fatty acids per day for six months — the daily equivalent of that found in about two cans of sardines. Controls received conventional care but no fish oil. Both groups were advised to eat a low-fat, low-cholesterol diet.

After six months, Milner and his colleagues evaluated their patients. "Our results showed a significant reduction in the clinical restenosis [closure] rate," Milner says. "The control group had a 35.4 percent reocclusion rate and the fish-oil group had only a 19 percent reocclusion rate."

That finding echoes an earlier study published in the Sept. 22 NEW ENGLAND JOURNAL OF MEDICINE. Gregory J. Dehmer of the Dallas Veterans Administration Medical Center and his colleagues found that 82 male patients treated with 5.4 grams of fish oil daily had a 16 percent rate of vessel renarrowing, compared with a control-group rate of 36 percent (SN: 9/24/88, p.197).

Fish oil gets a very different report card from Gregg J. Reis and colleagues at the Beth Israel Hospital in Boston. These researchers studied 186 randomly assigned angioplasty patients. Those in the treatment group got 6 grams of omega-3 fatty acids per day, while control patients received olive-oil placebo capsules. The researchers found that fish-oil patients had a 35 percent reocclusion rate, compared with 24 percent for controls. "We concluded that fish oil offered no protective effect in restenosis," Reis says.

Reis suggests Milner and Dehmer's results may be tainted by a defect in study design. In both of those studies, he notes, patients knew they were being given fish oil and researchers evaluating the vessel occlusion may have been unintentionally biased, tending to observe less vessel closure in fish-oil patients. In contrast, researchers and patients in the Beth Israel effort were unaware of who received the fish oil and who took the olive oil.

On the other hand, Reis' group may have introduced problems of its own by using olive oil as a placebo. Milner points out that olive oil is an unsaturated fat that may help prevent plaque buildup on artery walls.

Most researchers agree on the need for further study and a cautious approach to fish-oil treatment. Fish oil has drawbacks ranging from its "fishy taste" to possible bleeding complications. As for the current consumer craze, most experts say people are better off simply eating more fish dinners. *— K.A. Fackelmann*