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Making Insulin

A behind-the-scenes look at producing a lifesaving medication

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Operator Andrew McCoy monitors fermentors at the Eli Lilly & Co. insulin manufacturing site in Indianapolis.

Until the 1980s, trains brought piles of pig pancreases to Indianapolis from the surrounding pork farms. Workers unloaded the pinkish blobs into a building next to the railroad tracks that now serves as a parking garage in the shadow of Eli Lilly & Co., the first company to mass-produce insulin. Lilly miraculously turned those organs into a lifesaving medication.

In the days of pork insulin, it took more than 2 tons of pig parts to produce 8 ounces of purified insulin.

We've come a long way since more than 2 tons of pig parts were required to produce 8 ounces of purified insulin. Today, the insulin that comes in vials, pens, and pumps is not from pigs and cows but from designer microorganisms. These critters provide more of the hormone (and in forms more similar to the body's own) to the millions of people across the globe who depend on a steady stream of high-quality insulin.

The Birth of Big Biotech

Insulin, which is both a hormone and a protein, is a balled-up string of chemicals called amino acids. There are 20 common amino acids, such as tryptophan, that combine end to end to make proteins. Like the letters in a word, the order and number of amino acids in the protein are what define it. Human insulin's sequence of 51 amino acids differs from that of pork insulin by a single amino acid and by three amino acids from beef insulin.

The problem, though, is that the body's immune system is a fastidious fact checker, and it knows animal insulin is foreign. Some people with diabetes develop immune reactions to pork and beef insulin, and over time the insulin becomes less effective. And manufacturers needed a process that was more sustainable (and less stinky).

In the 1970s, scientists wanted to start making human proteins in the laboratory for their research and in hopes of developing new medications, and so they started tinkering with genes and organisms. Like proteins, DNA, the molecule that carries genetic information, is also a chain of chemicals—in this case, nucleic acids. By stringing together nucleic acids in the right order in the laboratory, genetic engineers found they could synthesize human genes, including the insulin gene. Each gene, a stretch of DNA, contains the instructions for making a particular protein. Scientists soon figured out how to insert a synthesized gene into a small loop of carrier DNA to aid in producing proteins. But it takes more than DNA to get a protein out of that gene. The ring of DNA needs to be inserted into an organism with all the biomachinery needed to assemble proteins.

In 1978, researchers at a burgeoning biotechnology company called Genentech announced that they had introduced a human gene for insulin into a safe strain of E. coli bacteria, which then produced the protein. Genentech partnered with Lilly, which brought engineered human insulin to the market in 1982 under the brand name Humulin, the first recombinant DNA drug product in the world. This biotechnology scheme is now in use worldwide for basic research as well as industrial applications, creating tens of thousands of protein varieties that help scientists to understand the human body and doctors to treat diseases such as cancer, rheumatoid arthritis, and, of course, diabetes.

Biotechnology allowed people with diabetes to take insulin that is virtually identical to the body's version. But that was just the beginning for insulin medications. Having the ability to tweak the insulin gene, scientists started to develop new-to-nature forms of insulin—called insulin analogs—such as insulin lispro (Humalog) and insulin glargine (Lantus). Analogs have become increasingly popular among prescribers and patients. Engineers build desirable properties into analogs by tweaking their amino acid sequences in ways that force the body to process them faster or slower than plain human insulin. These new attributes give people with diabetes more options for controlling blood glucose.

Quality Control

Insulin manufacturers are obsessed with quality control. At every step, someone or something makes sure that the insulin production is going smoothly. For example, after growing the bacteria, scientists at Eli Lilly & Co. check to make sure the insulin gene didn't mutate in the process. Sterility is also vital during growth, as one rogue pathogen could ruin a batch. Everything from the water to the air to the operators is held to the highest standards of cleanliness. Fionnuala Walsh, PhD, senior vice president for global quality at Lilly, said she'd be willing to undergo surgery on the floor of the growth room. It's that clean.

After every one of the many steps of purification, scientists check and double-check the insulin's purity. Even the packaging process is super-tightly regulated, as each vial of insulin is photographed from many angles while a computer program scans for trouble. The goal of an insulin producer, Walsh says, should be to provide a clean, consistent product so that people with diabetes can have one less thing to worry about.

From Few Come Many

For competitive reasons, pharmaceutical companies rarely give outsiders access to their manufacturing plants. But Lilly agreed to take Diabetes Forecast on a tour of parts of its Indianapolis insulin production complex. Covering 1.38 million square feet, or 18 soccer fields, the Lilly facilities in Indianapolis are constantly generating Humulin and the insulin analog Humalog, says Fionnuala Walsh, PhD, Lilly's senior vice president for global quality. Insulin producers Novo Nordisk and Sanofi follow broadly similar procedures for making their insulin analogs, though the details can vary significantly (see "Another Recipe," p. 57).

At Lilly, insulin-making E. coli is grown in 50,000-liter tanks called fermentors. There are more than 5,000 tanks on site.According to Lilly, a batch of insulin from one fermentor could produce a year's supply of insulin for thousands of people. "Our facilities are designed to produce insulin crystals in multiple metric-ton quantities," Walsh says.

The E. coli have humble beginnings. Small tubes of the bacteria have been stored in a freezer at minus 70 degrees Celsius (minus 94 degrees Fahrenheit) for decades. Lilly produced a granddaddy batch of E. coli, now referred to as the "master cell bank," sometime in the 1980s. It has gone on to seed every batch of Humulin to this day. Whenever Lilly wants a fresh stash of Humulin, workers go to the freezer, pull out a tube from the master cell bank, thaw it out, and stimulate the bacteria to grow.

Starting with a mere half gram of bacteria, the microorganisms begin to replicate prodigiously, doubling their numbers every 20 minutes or so. Once a tube gets too crowded, the bacteria are moved into larger and larger domiciles, from flask to bigger flask and from tank to bigger tank. All the microorganisms need to flourish is a source of water, sugar, salt, and nitrogen, which their handlers generously supply. In addition, the bacterial broth contains an additive that helps keep any contaminating microorganisms at bay, says Walsh. Typically, the E. coli are engineered to be resistant to a particular antibiotic, such as ampicillin, so that adding ampicillin to a broth will kill off everything but the prized protein producers. After several days of reproduction, the bacteria are now ready to start their real job—making insulin.

Until this point, the bacteria have been kept from making insulin by a repressor protein that sits near the insulin gene. To jump-start insulin production, the researchers free up the insulin gene by adding a chemical called an inducer to the giant vat of teeming bacteria. The critters

promptly begin to churn out insulin, holding the protein in clumps inside themselves. After a fixed period, typically a few hours, it's time for the harvest and the hard work of isolating the insulin from mounds of bacterial trash.

Another Recipe

OK, now that we've learned all that fun stuff about bacteria, let's look at another critter. Novo Nordisk, which started producing human insulin (Novolin) in 1987, employs a single-celled fungus: yeast. This microorganism, according to Jens Kirkegaard Baek, a Novo Nordisk specialist on insulin production, has certain advantages over bacteria when it comes to making insulin.

For starters, instead of trapping insulin inside themselves, as bacteria do, the yeast export insulin directly into the broth, says Baek, which simplifies purification. Another advantage of yeast over bacteria, he adds, is that yeast insulin is more mature to begin with and needs less wrangling with enzymes.

These yeast traits mean that Novo, instead of processing a big batch of yeast all at the same time, can continuously siphon yeast away from the large fermentors (above). As the yeast are removed and processed, the yeast's caretakers keep the fungi happily procreating by replacing the lost volume in the fermentor with more broth laced with nutrients. Once out of the fermentor, the yeast is easily separated from the now insulin-rich broth. Of course, there is still plenty of junk in the broth that needs to be removed before the insulin is pure and ready for patients, so Novo also utilizes purification columns to ensure the insulin is squeaky clean.

Clean Up

The first step in the purification scheme is to separate the bacteria from the broth. That's done with a centrifuge, a machine that spins very fast, forcing the bacteria into a pellet at the bottom of a vessel. The broth is then removed and replaced with a liquid containing a substance that breaks down cell membranes, helping release the insulin from its bacterial prison.

At this point, the insulin still isn't actually insulin. It's "proinsulin," a longer inactive precursor of insulin. Insulin makers use an enzyme to carve out a section of proinsulin, leaving behind just the 51 amino acids of insulin proper. (The part that is snipped out is called C-peptide. It's a hormone in its own right, and doctors sometimes measure it in the blood of people with type 1 diabetes to see whether their bodies are still making some insulin.)

The next phase of industrial purification involves an array of giant columns made of a clear material and filled with an opaque resin. Except for their size, the columns look much like

standard laboratory equipment. (This part of the Lilly production process was off limits, but the company showed this writer a model of a column.) When describing the girth of an industrial purification column, a smiling Lilly scientist stretched his arms out widely, bringing to mind an insulin-producing Parthenon. The columns are filled with various substances designed to separate insulin from other molecules based on differences in their electrical charge, acidity, size, and other characteristics. The insulin emerges from the columns alone.

At the end of its march through the mammoth columns, the insulin is quite pure. Yet, during processing, the insulin's chain of amino acids gets all tangled, rendering it inactive. To fix this, the researchers use yet another special mix of enzymes to iron out the wrinkles and get insulin into its proper form.

The final step before the insulin is ready for packaging is crystallization. The insulin is mixed with zinc, which helps it form stable crystals, and dried until it's nothing but a powder of glistening crystals. In due time, the crystals can be rehydrated in solution and poured into the vials, cartridges, and pens that are shipped around the globe.

Supply and Cost

For now, three companies supply all the insulin sold in the United States. Security at Lilly and other insulin plants safeguards the global insulin supply.

The cost of insulin is another common concern, but less expensive generic insulin may be on its way. Pharmaceutical companies may not be fans of more affordable generic versions of their products, but consumers love them—in 2009, 75 percent of all U.S. prescriptions were for generics. Until now, insulin makers haven't needed to compete with generic versions of their products. That protection may be coming to an end. Patents on insulin analogs, a growth area in the market, begin to expire in the next few years.

As the tour of the Lilly facilities reveals, making a biologic medication is complex, so generic insulins would probably not be made in exactly the same way as the name-brand versions. Small variations in production can alter the final product, and extra steps are needed to ensure consumers get what they pay for. The Food and Drug Administration (FDA) has outlined rigorous requirements for the approval of biosimilars, generic versions of medications, including insulin, that are made by microorganisms. These tests were developed to make sure biosimilars match the safety and effectiveness of the original products. So far, no generic insulin has been submitted to the FDA for clearance under the requirements adopted in 2012. Will generic insulins become an option in the future? Only time will tell, but with global insulin sales of $16.7 billion in 2011—a number expected to grow—companies have a financial incentive to give it a shot.

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