

From Cow Juice to a Billion Dollar Drug, With Some Breakthroughs in Between

by

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Part I – Introduction

Jack, a 15-year-old teenager, was playing Angry Birds on his iPad while his 85-year-old grandfather Fred was sitting nearby reading the newspaper and slowly dozing off.

“AHHHH, I got you, you dirty pigs!!! Don’t mess with the birds!” exclaimed Jack.

“Whaaaaat?!?! Cows? Eagles? What’s going on?” yelled Fred, as he awoke from his nap.

“I’m just playing my game, Grandpa. You try to hit pigs by shooting birds at them with a huge slingshot,” explained Jack with a mischievous grin on his face.

Fred became agitated as he started in on one of his well-rehearsed rants: “Baloney! You kids have no idea how good you have it these days! When I was a kid, for entertainment I would poke holes in the ground with a stick! And I had to walk three miles...”

“...in five feet of snow to school and back, yeah, yeah, yeah, I’ve heard that before,” interrupted Jack.

“It’s true!” proclaimed Fred. “As true as true can be! I bet you have no idea how different things were back then. Think about medicine! You know those insulin shots you get every day for your diabetes? Well, you know your grandpa here is diabetic too and things used to be a lot worse when I was your age. Your great-grandma used to inject me with cow juice to treat my diabetes! And sometimes it would make me feel really sick! My arm would hurt real bad for days, and sometimes it swelled up so much it looked like there was a golf ball under my skin!”

“That’s gross Grandpa, you’re totally making that up.”

“Why would I make that up?” asked Fred. “Your grandpa never lies! I was a boy scout when I was your age after all.”

“Really? This isn’t just another one of your stories like the one Christmas when all you got in your stocking was an orange and a pack of Lifesavers?”

“That orange was really small! And the Lifesavers were peppermint, not the sweet fruity ones! And did I ever tell you what I got for my seventh birthday? A single red crayon! And did I ever tell you about the time when ...”

As Fred rambled on endlessly about his childhood, Jack opened up Safari on his iPad and started a Google search for “history of insulin.” He found many interesting articles, and started reading.

Questions

1. What is diabetes? What are the different types of diabetes seen in humans?
2. What is insulin? Where is it produced? What is its physiological role? How does it carry out this function?
3. Which types of diabetes require insulin injections? How are other types of diabetes managed?

Part II – The Discovery of Insulin

Before insulin was discovered, being diagnosed with type 1 (insulin dependent) diabetes was a death sentence. Type 1 diabetes leads to high blood glucose levels (hyperglycemia), which can damage the kidneys, the brain, the cardiovascular system, the eyes (leading to blindness), and will eventually cause death. The only way to treat or manage diabetes was to eat a carefully controlled diet. One study of historical data concluded that a 10-year-old child diagnosed with type 1 diabetes during the years 1897-1914 had a life expectancy of only 1.3 years post-diagnosis.

Insulin was discovered in 1921 by Frederick Banting, John James Rickard Macleod, Charles Best, and James Bertram Collip at the University of Toronto. These scientists isolated secretions from the pancreases of cows and dogs, which colleagues referred to as “thick brown muck.” Insulin was then further purified from these secretions. Dogs that had their pancreases surgically removed exhibited significant levels of hyperglycemia, but after injection with the purified insulin, the dogs’ blood glucose levels dropped to normal levels. The scientific team was awarded the Nobel Prize in 1923 for their discovery of insulin and its actions on blood glucose levels.

The discovery by Banting et al. in 1921 led to the production of insulin as a therapeutic drug for diabetic patients. In 1922, the first clinical trials of purified bovine insulin began. The first person to receive bovine insulin was a 14-year-old boy named Leonard Thompson, whose body was ravaged by diabetes (he only weighed 65 pounds at the time). After receiving injections over the course of a few weeks, his condition improved dramatically and he was able to control his diabetes successfully. The pharmaceutical company Eli Lilly received approval to produce and sell purified bovine insulin to doctors and patients, which radically changed the lives of diabetics. During the period 1939–1945, the life expectancy for a 10-year-old child diagnosed with type 1 diabetes had improved to 45 years post-diagnosis.

Despite the successful treatment of diabetes with purified bovine insulin, adverse side effects were abundant. Injections sometimes resulted in abscess formation, pain, and swelling, in addition to potentially fatal allergic reactions. Since the purification process was not very robust, different vials of purified bovine insulin sometimes had different concentrations, which led to either over- or under-dosing effects, making it difficult to maintain an effective diabetes management program. It wasn’t until 1974 that improved purification methods were developed to prevent unintended side effects. Purified bovine insulin continued to be the primary source of insulin until the early 1980s.

Questions

1. Why was bovine insulin used as the source of insulin? Why not other animals? Consider pragmatic as well as biochemical reasons in your answer.
2. What are the major drawbacks of using insulin purified from animals?
3. How could you make human insulin in a lab? Give at least two methods.



Figure 1. Dr. Frederick Banting (right) and Dr. Charles Best, discoverers of insulin. *Source:* Library and Archives Canada, reproduction reference #C-001350.

Part III – Chemical Synthesis of Human Insulin – Close, but no Cigar

In order to meet the increasing demands for insulin, and to eliminate the adverse side effects of animal insulin, scientists began searching for ways to synthesize human insulin in the lab. The first attempts to produce synthetic human insulin made use of chemical reactions to join amino acids in the correct order to make the insulin protein. Before scientists could do this, however, they needed to know the primary amino acid sequence of insulin.

The amino acid sequence of human insulin was worked out by Frederick Sanger in 1951 (insulin was in fact the first protein to be sequenced), and he was awarded the Nobel Prize in 1958 for his discovery. Insulin is composed of two short chains, the A chain and the B chain, which are held together by disulfide bonds or bridges between cysteine amino acids present in the two chains.

Human insulin was chemically synthesized in the early 1960s by several academic groups, including those at the University of Pittsburgh and Aachen University in Germany. To do this, the scientists produced the A and B chains separately by joining the amino acids in the correct order through a series of chemical reactions, and then they mixed them together so that disulfide bonds could form. While the synthetic insulin was functional, the production process was very difficult, time intensive, expensive, and could not be scaled up to produce enough insulin to meet the needs of diabetic patients.

Another method was used by the pharmaceutical company Novo Nordisk A/S to chemically produce human insulin in the early 1970s. Scientists first purified insulin from pigs, which only differs from human insulin by a single amino acid. They then chemically treated the porcine insulin to change that single amino acid to the human amino acid, thereby producing human insulin. Novo Nordisk A/S marketed this product as “semi-synthetic” human insulin. However, other events that occurred during this time period led to the production of human insulin in a dramatically different and more effective way, as we shall see next.

Questions

1. What are some of the criteria a pharmaceutical company would want to meet if it is producing a drug for sale to consumers?
2. How do cells make proteins? Give a general overview of the process.
3. Do you think it is possible to exploit the biological process of protein synthesis to make human proteins in the lab? What would you need to do this? Describe the general process you would need to follow to accomplish this goal.

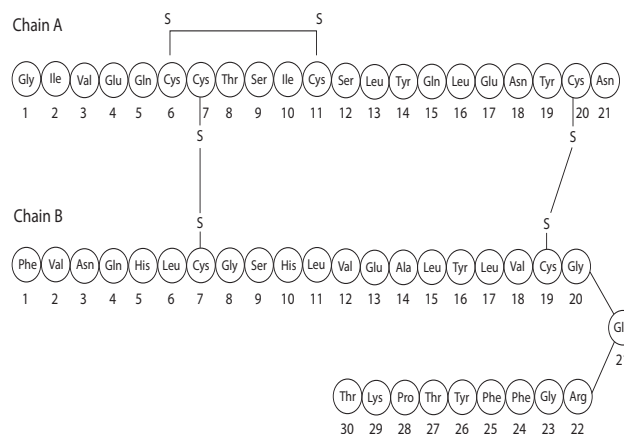


Figure 2. Amino acid sequence of human insulin. Each amino acid is represented by a circle. Disulfide bonds are shown by lines.

Part IV – Recombinant DNA Technology: The World Is about to Change

Scientific discoveries were being made in the 1960s and 1970s that would soon allow scientists to exploit the central dogma of molecular biology (DNA makes RNA makes protein) to produce any proteins they wanted in bacterial cells for further purification and use. They could use bacterial cells as mini “factories” to produce proteins from bacteria, plants, animals, and even humans. To achieve this remarkable process, scientists needed to be able to do four things:

1. Obtain the physical DNA molecule (gene) that encoded the protein of interest.
2. Insert that DNA into a bacterial DNA molecule (plasmid) thereby creating a recombinant DNA molecule (a DNA molecule composed of DNA sequences from two different organisms).
3. Insert the recombinant DNA molecule into the host organism (bacteria) so that the cells could make more copies of the DNA.
4. Grow the cells and have them to produce protein from the DNA.

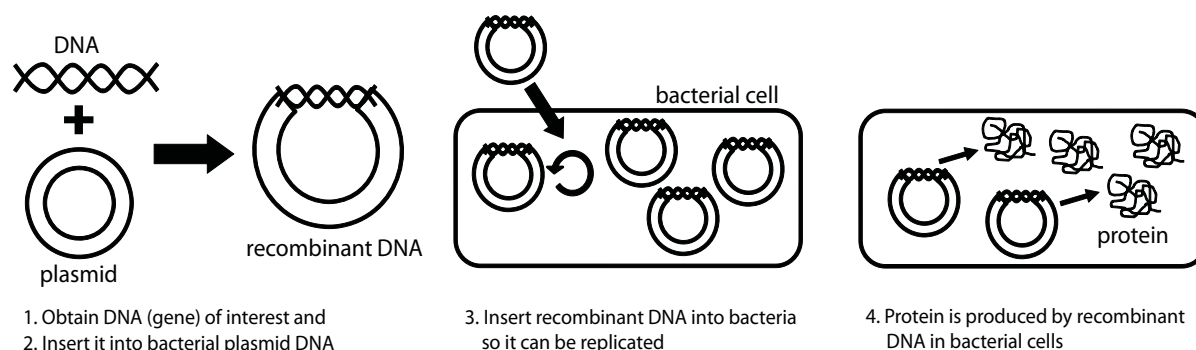


Figure 3. Basic steps in the recombinant DNA process.

These are the basic steps of recombinant DNA technology. Recombinant DNA is DNA from two different organisms that have been joined together in a single DNA molecule. Could this process be used to produce human insulin in bacterial cells? Could large enough quantities be produced so that it could be used to treat diabetes? Before these questions could be answered, several key developments in molecular biology had to be made.

The first recombinant DNA molecule was produced by Paul Berg at Stanford University in 1972. Berg inserted genes from the λ (lambda) bacteriophage (a type of virus that infects bacteria) into a mammalian virus, the SV40 monkey virus. He did this by “cutting” open both viruses using restriction enzymes (proteins that act like molecular scissors to physically cut DNA), removing genes from the λ bacteriophage, and inserting them into the SV 40 monkey virus. However, Berg stopped short of inserting this recombinant DNA molecule into living cells because of his concerns about potential biological hazards. Berg was awarded the Nobel Prize in 1980 for his work.

While Berg did not take that next step, Herbert Boyer (University of California at San Francisco) and Stanley Cohen (Stanford) did just that and thereby created the world’s first recombinant organism in 1973. Using restriction enzymes, they extracted DNA that encodes ribosomal RNA (rRNA) from the African clawed toad, *Xenopus laevis*. They then inserted these genes into plasmids, which are circular pieces of DNA commonly used by bacteria to produce proteins, yielding a recombinant DNA molecule composed of both toad and bacterial DNA. Next, they inserted this recombinant plasmid into living bacterial cells, and observed that the recombinant DNA replicated inside the bacteria and that the toad rRNA was transcribed. With this experiment, Boyer and Cohen discovered a way to insert recombinant DNA into other organisms and produce molecular products from it. This technology could possibly be used to produce proteins from other organisms in bacteria, potentially in large enough quantities to be used for medical therapies.

While the discoveries of Berg, Boyer, and Cohen would soon be used to produce recombinant proteins and change the world, there was significant fear from scientists (and the public) about the safety of recombinant DNA technology. In his research, Berg was using a monkey virus that could cause cancer; if he inserted it into bacteria and the bacteria infected people, would they get cancer? What if genes that produce toxic proteins were inserted into bacteria and those bacteria infected people? How should these recombinant organisms be safely contained? Should recombinant DNA research be allowed to continue without knowing the answers to these questions?

In 1974, Berg, Boyer, Cohen, and other scientists wrote a letter to the high-profile scientific journal *Science* that addressed the possible biological hazards of recombinant DNA technology. The letter, which came to be known as “The Berg Letter,” acknowledged “serious concern that ... artificial recombinant DNA molecules could prove biologically hazardous.” They proposed a moratorium (temporary stoppage) on all recombinant DNA research until potential hazards and safety measures could be addressed.

In 1975, the Asilomar Conference on Recombinant DNA, co-organized by Berg, took place in California where 140 scientists, doctors, and lawyers met to discuss the biological hazards and safety requirements necessary for working with recombinant DNA. The participants developed several guidelines for working with recombinant DNA, including the use of bacterial strains that would not survive outside of the lab and a series of risk levels that required specific types of containment and safety protection. After the conference, the research moratorium, which was voluntarily observed for eight months, was lifted and recombinant DNA research flourished. In 1977, Boyer and his colleagues used recombinant DNA technology to produce recombinant human somatostatin, a 14-amino acid neurotransmitter that regulates the secretion of growth hormone, as a proof-of-principle experiment to show that human proteins can be produced in bacteria. At this point, all of the tools were in place to make recombinant human insulin in bacteria for use as a pharmaceutical drug.



Figure 4. Photo of Paul Berg, National Library of Medicine, public domain, <http://profiles.nlm.nih.gov/CD/B/B/L/L/>.

Questions

1. What properties of bacteria make them attractive for producing recombinant proteins? Are there any properties that are unattractive? Specifically address biochemical processes.
2. Do you agree that the Berg Letter, research moratorium, and Asilomar Conference were necessary before further recombinant DNA research was conducted? Why or why not?
3. Make a sketch to show the process by which human insulin could be made by bacteria using recombinant DNA technology.

Part V – The Production of Recombinant Human Insulin – The World Has Changed

The stage was set for scientists to produce recombinant human insulin using recombinant DNA technology. There was in essence a “race” between two competing groups to be the first to produce recombinant human insulin.

One group was composed of scientists at Harvard University and a private company Biogen, and the other was made up of scientists from The City of Hope Medical Research Center (Pasadena, CA) and a new biotechnology company, Genentech, co-founded by Herbert Boyer. Biogen and Genentech both used different methods to produce recombinant human insulin, and ultimately only one company was successful.

Since the first step in creating a recombinant protein is obtaining the gene that encodes that protein, Biogen scientists aimed to isolate the insulin gene from human DNA samples by using rat insulin DNA as a probe. The rat insulin gene had been previously isolated and its sequence was very similar to that of the human insulin gene. The rat insulin gene was radioactively labeled and used to probe human DNA samples (since the sequences were similar it would bind to the human insulin gene). The human insulin gene was then identified, isolated, and introduced into bacterial cells for production of human insulin. However, the Biogen scientists were not successful at producing recombinant human insulin using this method, as recurrent contamination with the rat insulin gene resulted in production of recombinant rat insulin.



Figure 5. Herbert Boyer, co-founder of Genentech. *Source:* Gitschier J. (2009) “Wonderful Life: An Interview with Herb Boyer.” *PLoS Genetics* 5(9): e1000653. doi:10.1371/journal.pgen.1000653. Copyright: © 2009 Jane Gitschier, CCAL.

Genentech used a different strategy than Biogen and ultimately was successful. Since the sequence of human insulin was known, instead of isolating the human insulin gene from DNA samples, scientists at Genentech chemically synthesized the human

insulin gene in the lab. They then inserted the human insulin gene into a plasmid, added it to bacteria, and produced recombinant human insulin in these bacterial cells. This strategy was successful and in 1978 Genentech became the first company to produce human insulin using recombinant DNA technology.

Recombinant human insulin could now be produced in large quantities easily and did not produce the adverse side effects that animal insulin did. Genentech licensed their production process to Eli Lilly and Company (the same company who sold the first animal insulin back in 1922!) and the world’s first recombinant therapeutic drug was born: Humulin.

In 1982, Eli Lilly and Company introduced Humulin to doctors and diabetic patients after passing clinical trials and earning approval from the Federal Drug Administration. Humulin has essentially completely replaced bovine insulin as the primary treatment for type 1 diabetes today, and in 2011 alone, Eli Lilly and Company made \$1.25 billion in revenue from sales of Humulin. The company made another \$2.37 billion in revenue in 2011 from sales of a recombinant insulin analog, Humalog (two amino acids are changed, which increases the ability of the body to use the recombinant insulin).

Since the introduction of Humulin, many more recombinant protein drugs have been developed, including human growth hormone, erythropoietin, and parathyroid hormone. Recombinant DNA is now not only used to produce recombinant proteins to be used as drugs, but also to make genetically modified organisms (GMOs). Such GMOs include animals with higher lean muscle content and plants resistant to herbicides and/or pesticides, and organism used to make enzymes for industrial processes. Recombinant DNA technology and recombinant proteins are also commonly used by scientific research groups world-wide in biological and biomedical research.

Questions

1. Why do you think Genentech's method to produce recombinant insulin worked while Biogen's method did not?
2. What are the advantages of using recombinant human insulin to treat diabetes over using purified animal insulin?
3. If you wanted to design and produce a recombinant protein other than insulin, what protein properties do you need to take into consideration?
4. What do you think the world would be like today if we couldn't make recombinant proteins? What products that we take for granted wouldn't exist?

Part VI – Conclusion

“And there was that summer when I was 14, and I delivered newspapers. I got one nickel a day! One nickel!” Fred continued to drone on.

“What are you talking about Grandpa?” Jack said as he looked up from his iPad. “I’ve been reading about how our insulin medicine was made. I guess you were telling the truth, they really did use cow juice to treat diabetes. But then some really smart scientists figured out a better way!”

“See! Your grandpa always tells the truth! Scouts honor!” exclaimed Fred.

“I still don’t believe that you had to walk three miles in five feet of snow to get to school though, you are definitely making that one up,” said Jack.

“Of course I did!” exclaimed Fred. “I’ll tell you all about it again, but before I do let me try out that Happy Snakes thingamajig you got there.”

“It’s called Angry Birds, Grandpa...” Jack said as he shook his head slowly.



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