Tom Layloff

Insulin: The Wonder Drug

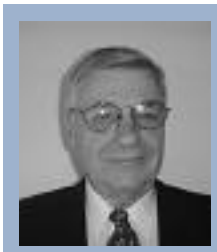
A STORY OF DOGS, COWS, PIGS, BUNNIES, AND US*

The health professions and national drug regulatory agencies (i.e., the FDAin the U.S.) are passing through a transition from drugs of relatively low molecular weight that can be assayed by the traditional tools of the analytical chemist to drugs whose chemical structures are not always well understood or whose molecular weight is much higher than we analytical chemists are accustomed to. In addition, many of the newer products are produced in large part by fermentation rather than in traditional chemical reactors. In reflecting on this transition, recombinant human insulin presents an interesting case. It is a biotechnology-based continuation of the story of an over 80-year-old therapeutic family of chronic-use, invasively delivered wonder drugs that have been subject to FDA regulation for over 50 years. The evolution of both the product and the quality assessments provides one of the most fascinating stories in the drama of sickness interventions.

Scientific fidgeting with insulin began in earnest in the late 19th century, but the breakthrough occurred with Frederick Grant Banting and Charles Herbert Best in their seminal work on ligating the pancreatic duct of a dog in 1921, thereby causing the pancreatic tissue to degenerate. This facilitated the extraction of the antidiabetic factor from the islets of Langerhans.** The extract thus obtained was injected into a vein of a dog whose pancreas had been removed to induce a diabetic state. That dog became more active and its blood sugar levels reduced. This experiment was repeated on another dog, which confirmed Banting's hypothesis that the pancreatic tissue could be caused to degenerate, thereby reducing degradation of the antidiabetic factor we call insulin. To establish a more reliable source for the insulin used in his experiments, Banting, based on his experience on a cattle farm, shifted to the fetal calf pancreas in 1922. Fortunately, this new source also yielded an extract that, upon injection into induced-diabetic dogs, dramatically reduced blood sugar levels. The success of these experiments with dogs led to the first human experiment, an injection of bovine extract insulin into a teenage patient who was in a diabetic coma. Again, the extract ameliorated some of the diabetes symptoms. Based on this success, further human studies were initiated, which showed that in addition to the antidiabetic effects, abscesses formed at the injection sites, presumably due to impurities. In addition to the abscesses, there were other undesirable side effects, also presumably due to impurities. Efforts to purify the insulin factor to reduce these side effects were undertaken by a biochemist col-

*This paper is based in part on materials published at www.discoveryofinsulin.com; Kahn EJ Jr. All in a century: The first 100 years of Eli Lilly and Company, 1975, **Eli Lilly ,** Indianapolis, IN; www.pbs.org/wgbh/aso/databank/entries/dm22in.html; and Crystallographic studies of modified insulin, Maria Gertrudis Wilhelmina Turkenburg-van Diepen, Ph.D. Thesis, Dept. of Chemistry, University of York (U.K.), Sept 1996 (www.yorvic.york.ac.uk/~mgwt/thesis-tth/chapter1.html).

**Banting operated on one of the duct-tied dogs and found that the ligature had held and that the pancreas had shrunk to about one-third of its normal size. The gland was removed, chopped up and ground in a mortar with saline, strained, and a small amount injected into a vein of a depancreatized or diabetic dog (www.discoveryofinsulin.com).



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league, J.B. Collip, in 1922. In addition to significantly improving the purity of the factor to reduce the abscess formation and side effects, Collip, while working with rabbits, observed that too much of the factor could induce hypoglycemia, which also could provide the basis for a bioassay.

Because of their concerns that the insulin production process could be pirated, resulting in spurious and substandard insulin in commerce, Banting and Best patented their discovery and assigned it to the University of Toronto (Toronto, Canada) to protect the public interest and health. This precedent-setting act set the stage for the eventual deluge of academic institutions intervening to patent faculty inventions. The Board of Governors of the University created the Insulin Committee to handle licensure of the patent and oversee quality control of the product. When the insulin patents expired, the U.S. Congress passed legislation requiring the continuing certification through the Insulin Committee* of batches of

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bulk insulin intended for marketing in the U.S., and also established lot-by-lot product testing and certification by the FDA. The Insulin Committee continued to certify bulk lots of insulin drug substance for the U.S. market until the 1970s when this function was assigned to the FDA along with their program of lot-by-lot product certification. The FDA continued the certification of all lots of insulin bulk drug and products until the U.S. Congress terminated the program in 1997. The program termination was a recognition that insulin had graduated from an extract defined through potency bioassays and surrogate assays to well-defined chromatographic chemical assays. Over the years the improved product quality followed the evolution of improved assessment and purification technologies, especially column chromatography and its successor, HPLC.

In 1922, Banting and Best accepted an offer from **Eli Lilly Co.** (Indianapolis, IN) to collaborate to improve the purification, concentration, and stability of the extract. This collaboration was very successful, and a markedly improved supply was available for further clinical studies. The insulin source was initially bovine based but later porcine extraction was also included. This production eventually led to the large-scale manufacture of insulin, which by the late 1980s required virtually the entire available stock of beef and pork pancreas produced in the U.S. It is striking to reflect that insulin was in use for approximately six years before scientists established it was a protein, and it took yet another generation for the primary structure of bovine insulin to be elucidated. Collip's observations that insulin-induced hypoglycemia in rabbits became the basis of assigning the potency of insulin preparations, a great breakthrough in quality control of this life-saving wonder drug.

Table 1

Insulin A and B chain amino acid differences

Source	A-8	A-10	B-30
Us	Threonine	Isoleucine	Threonine
Cow	Alanine	Valine	Alanine
Rabbit	—	—	Serine
Pig	—	—	Alanine

We now know that bovine and porcine insulins differ from human insulin in their amino acid sequences and that rabbit insulin also differs from the other three. Those observations were published about 60 years after the initial discovery of the antidiabetic factor in dogs. (See *Table 1* for the differences.[1])

The potency assessments for human use of the bovine- and porcine-derived insulins were performed using rabbits. Fortunately, the human and rabbit insulin receptor sites are relatively robust and insensitive to B-30 substitution, so the test worked. It also is very helpful that this small protein molecule is not terribly antigenic, so again this worked for chronic use in rabbits and humans.

The product quality assessments used in U.S. Pharmacopeia XII[2] states, "Insulin Injection is an acidified aqueous solution of the active principle of the pancreas which affects the metabolism of glucose. Insulin Injection, when assayed as directed, shall possess a potency of not less than 95 per cent and not more than 105 per cent of the potency stated on the label, and the potency shall be expressed in U.S.P. Insulin Units which are equivalent in potency to the Unit declared on the label of the container of the *U.S.P. Zinc-Insulin Crystals Reference Standard*. Insulin Injection is so standardized that each cc. contains either 20, 40, 80, or 100 U.S.P Insulin Units." The product identification was the rabbit hypoglycemia convulsion test and the surrogate assessment panel for the human use product included total nitrogen, zinc, ash, and rabbit bioassay.

The bioassay of the bulk insulin drug substance was tied to the early master lots and through the zinc-insulin crystals and eventually to "pure" reference standard materials. The current USP 24[3] requires for tissue-derived insulin drug substance not less than 26.5 USP insulin units in each mg and not less than 27.0 USP insulin units for the material labeled as purified. This should be compared to the theoretically pure material of ca. 28. For the human insulin product the USP 24 requires not less than 27.5 insulin

*It now appears quite extraordinary that the U.S. Congress would assign responsibility for the quality certification of insulin for U.S. commerce to a foreign body. However, this should be considered in light that Congress also assigned legal responsibility for pharmaceutical product quality in 1906 to the U.S. Pharmacopeia, a not-for-profit independent association. This also is consistent with the regulatory mutual recognition built between the U.S. and Canada through the then Association of Official Analytical Chemists, which also established product standards.

units in each mg. The current USP 24 limits the proinsulin levels in tissue-derived insulins to not more than 10 ppm and applies this same limit for the host-cell-derived proteins for the recombinant DNA source materials.

One cannot help but be struck by how remarkably the technologies have changed the product quality. In the early part of the FDA certification program, insulin bulk drug substance had been certified with an assay as low as ca. 22 units per mg. Of course, these less pure materials likely caused many side and antigenic effects in patients with diabetes who used this product, while they added remarkably to the quality and length of the lives of individuals with diabetes. Of course, the continuing saga of purification, crystallization, development of longer-acting formulations, etc., which spanned over three generations of patients with diabetes, is one of an evolution of purification and assessment technologies. These research and purification efforts culminated with the synthesis of the gene for human insulin in 1978 and its insertion into bacteria to create the first genetically engineered pharmaceutical product. This synthesis freed patients from the specter of chronic injection of an animal-tissue-derived product and allowed the use of essentially pure human insulin.

For this publication, the presentation speech by Prof. A. Tiselius for Frederick Sanger's award of the 1958 Nobel Prize in Chemistry is especially noteworthy (http://nobel.se/chemistry/laureates/1958/press.html).

> "The proteins are among the most complicated and enigmatic substances in Nature and appear to be particularly closely related to all that we call Life. To this group of key substances belong for example all enzymes and many hormones, which control the chemical processes of Life, also the viruses and many toxins which cause disease, and antibodies, which are formed on vaccination, and are able to protect us against infection. In blood and in all tissues of the body, in muscles, nerves and skin, proteins form an essential and functional constituent. It is the chemical individuality of proteins which is responsible for the species' differences among all living things. *The determination of the exact building-plan for these complicated giant molecules appears as one of the greatest problems in today's scientific research* [italics added by author]. Even if some protein molecules are big enough to be observed in our most powerful electron microscopes it is not possible by any direct method to see the finer details in their structure. Here one must resort to the indirect methods which chemists use in studying the structure of complicated substances. Thus one has to break down the big molecules by suitable methods and look for simpler and well-known substances among the fragments obtained. This procedure was used with proteins by the German chemist and Nobel laureate Emil Fischer in the beginning of this century. He found that protein molecules contain long chains of so-called amino acids. These are comparatively simple substances of which about 25 different kinds are found in Nature, and they are formed when proteins are boiled with strong acids. Thus we know that proteins contain a large number of different kinds of amino acids, but the composition and, above all, *the sequence* [original italics], of the amino acids in the chains vary considerably. As a matter of fact it has long been assumed that it is this sequence which determines the individual chemical and physiological properties of different proteins.
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> That insulin is a physiologically important hormone which is used in the treatment of diabetes is well known to all. Insulin is also a protein and even if its molecules do not belong to the largest, they are sufficiently complicated to make the task of determining their structure appear a formidable one. It was this venture, however, which Frederick Sanger started fifteen years ago and which after zealous and persistent work gradually led him to a successful solution of the problem; namely the exact mode in which the 51 amino acids of the insulin molecule are linked together."

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Reflecting on Prof. Tiselius' 1958 remarks, it is striking to see that although we have come a long way, these words are just as pertinent now as they were over 40 years ago. The sequence issues still remain. The complexities arising from posttranslational modifications and the exquisite tertiary structures and quarternary aggregates in living systems are not yet within near reach. Our technologies and information systems have indeed brought us a long way over the 80 years since the antidiabetic factor was discovered, but I think the journey has just begun.

References

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