The History of Insulin

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THE PREHISTORY OF INSULIN — Insulin received its name before it was discovered. In 1889 in Germany, Oskar Minkowski and Joseph von Mering observed that total pancreatectomy in experimental animals leads to the development of severe diabetes mellitus, and began the speculation that a mysterious substance produced by the pancreas is responsible for metabolic control (1,2). Supporting evidence for the hypothesis gradually mounted: It included observations of the relationship between diabetes and damage to the pancreatic cellular system known as the islets of Langerhans, as well as the discovery and elucidation of the physiology of internal or endocrine secretions. By the first decade of the 20th century it was widely hypothesized that an "internal secretion" of the pancreas controls carbohydrate metabolism (3).

But no one could demonstrate that the internal secretion actually existed. Minkowski himself was the first of innumerable researchers and physicians who administered pancreas solutions, orally and by injection, to diabetic animal and human subjects in the hope of replacing the missing substance. Results were decidedly mixed and inconclusive. Experiments in which extracts of the pancreas appeared to reduce glycosuria often were unrepeatable or marred by strange patterns of fever and other reactions. With the wisdom of hindsight, we now know that many of these experimenters, such as Georg Zuelzer in Germany or D.A. Scott in the U.S., were, in fact, administering active insulin. But it was impossible to present clear evidence of benign hormonal action because of so many toxic contaminants in their preparations. Beginning in 1906, for example, Zuelzer occasionally was able to reduce glycosuria and acidosis in human diabetic individuals; but the results were accompanied by such severe, life-threatening reactions that workers in Minkowski's laboratory dismissed his work as inconclusive and dangerous (4).

Even so, there was so much impressionistic evidence supporting the existence of a pancreatic internal secretion, emanating from the islet cells, that, in 1909, a Belgian investigator, J. de Meyer, proposed it be named "insuline" (5). In 1916, E.A. Schafer in Britain independently suggested the same name (6). Much truth is in the notion, again clarified by hindsight, that insulin was sitting there waiting to be isolated or "discovered." It almost certainly would have been found during the second decade of the twentieth century, but the work of Central European researchers, such as Zuelzer and the Romanian physiologist, N.C. Paulesco, was utterly disrupted by World War I.

On the other hand, careful students of carbohydrate metabolism knew that there might be other explanations (relating to the nervous system or other pancreatic mechanisms) for the relationship between pancreatic failure and diabetes. And experimentation had been maddeningly imprecise because of the difficulty of measuring the physiological effect of various interventions. The secret of the pancreas could not be uncovered without better tools.

The development of methods that permitted rapid serial readings of blood glucose levels was a precondition for the eventual breakthrough. By 1919, an advanced researcher, Israel Kleiner, working at the Rockefeller Institute, was able to show that intravenous injections of aqueous solutions of ground fresh pancreas had a regular hypoglycemic effect. Only a pattern of slight toxic effects of his extracts prevented him from attempting the administration to human diabetic individuals, which, if successful, would be judged real proof of discovery. Unfortunately for Kleiner, he left the Rockefeller Institute that year for a university that did not have the resources to support major animal research and he abandoned the work. Other investigators, such as Paulesco in Romania, were making very slow progress because of inadequate funding and hopelessly primitive experimental techniques (7,3).

BANTING'S RESEARCH — In 1920, Frederick Grant Banting was a 22-yr-old physician and surgeon attempting to launch a general practice in the small Canadian city of London, Ontario. With time on his hands, he accepted a demonstratorship in surgery and anatomy at London's Western University. On Monday, 31 October, he had to talk to physiology students about carbohydrate metabolism, a subject with which he was not particularly familiar. Late Sunday night, as part of his preparation, he read the leading article in the November issue of *Surgery, Gynecology and Obstetrics*, a discussion of "The Relation of the Islets of Langerhans to Diabetes with Special Reference to Cases of Pancreatic Lithiasis," by Moses Barron (8). Barron's unremarkable report stimulated a train of thought in Banting's mind that caused him, sometime after midnight, to jot

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down this idea: "Diabetus Ligate pancreatic ducts of dog. Keep dogs alive till acini degenerate leaving islets. Try to isolate the internal secretion of these to relieve glycosurea" (9).

Banting enjoyed dabbling in research and was unhappy in his fledgling practice. He returned to his alma mater, the University of Toronto, and approached J.J.R. Macleod, professor of physiology, with a proposal to engage in summer research to test his "Diabetus" idea. Macleod, a noted expert in carbohydrate metabolism, doubted that a novice could succeed where masters had failed. However, he may have seen some value in Banting's hypothesis that the internal secretion was somehow being nullified in pancreatic extracts by the action of the externally secreted digestive ferments. By ligating the pancreatic ducts, Banting hoped to induce atrophication of the acinar cells and eliminate the external secretion, thus liberating the internal secretion. Banting's training as a surgeon would serve him well in such research; it also predisposed him to an interest in grafting experiments as the second stage in his work. In an age before the rejection phenomenon was understood, several experts had suggested pancreatic grafts or transplants as a promising direction in the search for the elusive secretion. With surplus facilities at hand in his very well-equipped laboratory, Macleod agreed to give Banting space, dogs, and a student assistant for a summer "fling" at the problem. One of Macleod's summer students, Charles Best, won a coin toss to see who would start work with Banting (3).

Banting began his research, assisted by Best, on 17 May 1921. Macleod was both the formal supervisor and an active adviser before leaving the city in mid-June. The casualty rate among Banting's dogs was high, some depancreatized, others duct-ligated. At the end of July, he and Best began intravenous injections into depancreatized animals of saline extracts of chilled atrophied pancreas. They observed a pattern of hypoglycemic effects. When Macleod returned in September, he urged Banting and Best to repeat and amplify their experiments. He discouraged Banting from venturing down the grafting road and, after some friction with the young doctor, supplied more space and dogs.

By December, Banting and Best had accumulated further evidence that their extract often reduced the blood glucose of diabetic dogs. After experiments with fetal calf pancreas and then with fresh beef pancreas, Banting found he could dispense with the cumbersome duct-ligation/atrophication procedures (though he never quite realized that in doing so he had disproven his original hypothesis of an antagonism between the pancreatic secretions). Because of Best's inexperience, Macleod and Banting decided to add J.B. Collip to the research team. Collip, a biochemist from the University of Alberta, was visiting Toronto to work with Macleod and had expressed an interest in the pancreas work.

The first presentation of the Toronto research, read at the New Haven meeting of the American Physiological Association on 30 December 1921, was not well received. In their inexperience and haste, Banting and Best had been sloppy and muddled. Their lack of data on the side-effects of their extracts, for example (which were almost certainly pyrogenic, as others had been), meant that it was difficult to convince anyone that their findings were better than those of Kleiner and others. The team's most recent experiments, notably evidence compiled by Collip on the extract's apparent restoration of glycogen mobilization in the liver and its ability to clear ketones, may have seemed more promising (3,10).

THE DISCOVERY OF INSULIN — On 11 January 1922, clinicians at Toronto General Hospital injected a 14-year-old, severely diabetic boy, Leonard Thompson, with 15 ml of pancreatic extract made by Banting and Best. This clinical test was a failure. The injection caused only slight reductions of glycemia and glycosuria, had no effect on ketoacidosis or the patient's subjective presentation, and resulted in the formation of a sterile abscess. "These results were not as encouraging as those obtained by Zuelzer in 1908," Banting later wrote. Treatment was immediately discontinued (11).

On January 23, a new series of injections began. Thompson responded immediately. His glycosuria almost disappeared, his ketonuria did disappear. His blood glucose dropped to normal. He was brighter and stronger. For the first time in history there was clear, unambiguous evidence that scientists were able to replace the function impaired in diabetes. This was the demonstration of the isolation of the internal secretion of the pancreas that the world had awaited for 30 years.

It was J.B. Collip, the biochemist, who had produced the successful extract. He had developed a method of extraction that involved changing the concentrations of slightly acidic alcohol solutions of chilled beef pancreas (it is not clear which members of the research team first suggested using acid alcohol) until he was able to precipitate out the active principle relatively free from toxic contaminants. It was a major improvement on Banting and Best's methods, the single most important step forward in the discovery process (12,13).

Unfortunately, the triumph was marred by bitter personal rivalries; Banting and Best believed that Collip and Macleod were conspiring to take over control of the work and credit for its success. Physical and verbal confrontations often disrupted the research. Face-saving agreements, such as a decision to publish alphabetically, barely camouflaged intense personal dislikes. Bitter controversy about credit for the discovery of insulin scarred Toronto's great achievement until the last of the researchers, Best, died in 1978. Banting and Best were particularly confused and self-serving in their refusal to recognize their collaborators' contributions to the

work, in refusing to recognize as Llewelyn Barker put it, that "in insulin there is glory enough for all" (3).

The glory came almost immediately. On 3 May, 1922, Macleod delivered a complete summary of the Toronto work at the Washington meeting of the Association of American Physicians. By now it had been decided to name the active principle "insulin." Macleod suggested the Latin root for islands without knowing of Meyer's and Schaeffer's earlier proposals. The audience agreed that the Toronto team had made one of the great breakthroughs in modern medicine and gave them a standing ovation (14). Eighteen months later, in one of the fastest recognitions of a medical discovery in its history, the Nobel Committee of the Caroline Institute awarded the 1923 Nobel Prize in physiology or medicine to Banting and Macleod. Banting divided his prize money equally with Best; Macleod split his with Collip. The Nobel Committee was probably mistaken in not having named Collip as a co-recipient of the prize.

THE DEVELOPMENT OF INSULIN— The Toronto team was attempting to extract an unknown substance from the pancreas with what we would now consider primitive equipment. It was unable to produce insulin in anything but laboratory batches, and even these were often contaminated, weak, or completely ineffective. After many frustrating failures in the spring of 1922, it was decided to undertake a joint venture with Eli Lilly and Company of Indiana, a well-established, ethical drug company whose research director, G.H.A. Clowes, had expressed persistent interest in the work. A formal agreement was signed on 30 May, 1922, and Best and Collip went to Indianapolis to share all the formulas. By the end of June, Lilly was producing potent preparations of porcine insulin, which were shipped to Toronto for testing.

The most important breakthrough in insulin development was made that autumn when Lilly chemists, simultaneously with and independently of a research team at Washington University in St. Louis, discovered a method of producing large quantities of much purer insulin through isoelectric precipitation. In early 1923, Clowes boasted of Lilly's capacity to produce enough insulin "to supply the entire needs of the civilized world." In point of fact, the Toronto group had granted Lilly only a one-year exclusive license on insulin production for the United States and Latin America. To solidify control of the discovery, the researchers had assigned patents on their methods to the University of Toronto, which licensed manufacturers in other countries—including its own Connaught Laboratories in Canada—as well as competitors in the United States.

One of the first Europeans to visit Toronto to learn about insulin was August Krogh, a Danish Nobel laureate, who we now know was eager to obtain insulin to treat his wife's diabetes. Aided by a brilliant chemist, H.C. Hagedorn, Krogh began insulin production in Copenhagen early in 1923 at their Nordisk Insulin Laboratory. All of the pioneering manufacturers faced immediate, immense problems involving standardization of the product, dosage, diet, physician and patient education. On the other hand, there were, as yet, no government regulatory hurdles to overcome. It is a testimony to the idealism and efficiency of both the University of Toronto group and the leading manufacturers that, by the end of 1923, insulin was being used commercially and safely to treat people with diabetes in most Western countries. The two major producers, Lilly in the United States and the Danes in Europe (Novo Company developed as an early breakaway group from Nordisk), used their head start in insulin knowledge to begin to build a global dominance in insulin manufacture that remains today, and so, gloriously, do many of the patients first treated with insulin in the early 1920s.

THE MULTIPLICATION OF INSULINS— At first, it was hoped that insulin could be delivered orally, and/or that a more fundamental hypoglycemic substance, perhaps contained in plants, remained to be isolated. For almost a decade, there was dispute over insulin's chemical composition. Manufacturers gradually improved the purity of the product; and, in 1926, J.J. Abel at Johns Hopkins was able to crystallize insulin. In the next few years it was finally accepted that the hormone is a protein. The study of insulin would have a great impact on protein chemistry over several decades (15). In the meantime, manufacturers strove to develop insulin compounds that would most effectively meet the requirements of users for easy administration and closer matching with physiological need.

The first Toronto patients received one injection a day of an extremely impure insulin. As "regular" bovine and porcine insulin was increasingly purified in the early years of manufacture, patients complained of the inconvenience of having to take several injections a day. A search began to prolong the action of insulin by combining it with other substances. In the mid-1930s, Hagedorn in Denmark discovered that basic proteins, notably protamine, when added to insulin could prolong its action. In Toronto, Scott and Fisher simultaneously learned that zinc also had a lengthening effect. These discoveries paved the way for a gradual multiplication of insulin products in the late 1930s and 1940s. Protamine-zinc insulin (PZI) jostled onto the marketplace with protamine- or isophane-insulin (NPH), and soon combinations of the long-lasting and regular insulins were available. In the mid-1950s, Novo pioneered the introduction of the lente insulins, which contained zinc but not protamine. Innumerable mixtures of quick-acting, intermediate, and long-acting insulin were now possible. Many patients now took insulin only once a day, but many others

seemed to do better on multiple doses (16).

In the 1970s, yet another generation of animal insulins was introduced as a result of new processes aimed at eliminating proinsulin and other immunogenic peptides. These "monocomponent" insulins took the preparation of pure insulin from animal pancreases about as far as it could go. Throughout all these years, it was remarkable that the animal pancreas supply had never been a problem in the developed countries. However, some countries had occasional supply shortages during World War II. The more common reasons for insulin not being available to people with diabetes, one suspects, were disorganized manufacture in Third World and communist countries, and missed diagnosis by physicians everywhere.

TOWARD A NEW ERA— Insulin was given to the world as a result of messy, confused, experimentation on living subjects. It was the mysterious, magical secretion of the pancreas that researchers finally learned how to extract from animal pancreas—removed at the abattoir immediately after slaughter—in forms suitable for administration to humans.

By the late 1950s, chemists understood the exact structure of the insulin molecule in the context of a dazzling explosion in our knowledge of DNA and the processes of life itself. Within another twenty years, what had once seemed a wild science-fiction dream, the idea of manipulating genes to create life forms in the laboratory, was now a practical possibility. The great scientific revolution of our time—the advent of molecular biology—made it possible to conceive of genetic engineering techniques that could lead to the biosynthesis of real human insulin.

The era of animal insulins, as they had become life-saving, creatively compounded, and beautifully purified, drifted toward its end.

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