Insulin: A 100-Year-Old Discovery With a Fascinating History

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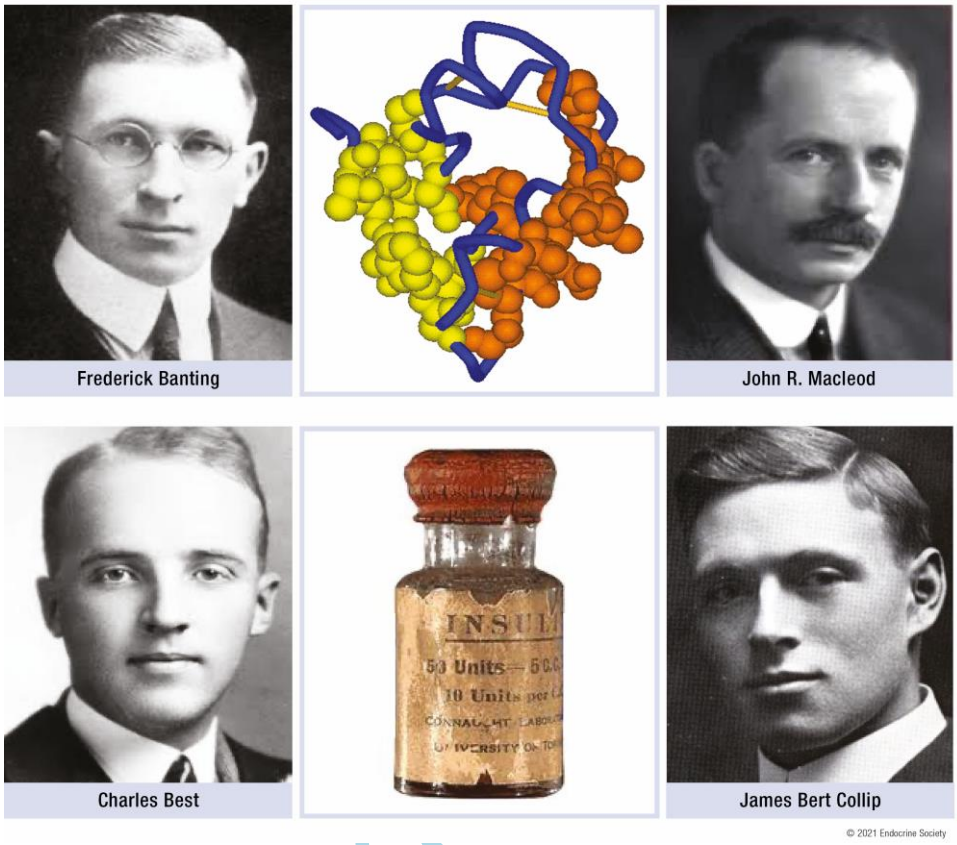
Abstract

Diabetes has been known since antiquity. We present here a historical perspective on the concepts and ideas regarding the physiopathology of the disease, on the progressive focus on the pancreas, in particular on the islets discovered by Langerhans in 1869, leading to the iconic experiment of Minkowski and von Mering in 1889 showing that pancreatectomy in a dog induced polyuria and diabetes mellitus. Subsequently, multiple investigators searched for the active substance of the pancreas and some managed to produce extracts that lowered blood glucose and decreased polyuria in pancreatectomized dogs, but were too toxic to be administered to patients. The breakthrough came 100 years ago when the team of Frederick Banting, Charles Best and James Collip working in the Department of Physiology headed by John Macleod at the University of Toronto managed to obtain pancreatic extracts that could be used to treat patients and rescue them from the edge of death by starvation, the only treatment then available. This achievement was quickly recognized by the Nobel Prize in Physiology or Medicine to Banting and Macleod in 1923. At 32, Banting remains the youngest awardee of this Prize. Here we discuss the work that led to the discovery and its main breakthroughs, the human characters involved in an increasingly dysfunctional relationship, the controversies that followed the Nobel Prize, and the debate as to who actually "discovered" insulin. We also discuss the early commercial development and progress in insulin crystallization in the decade or so following the Nobel Prize.

Keywords

Diabetes, insulin, pancreas, discovery of insulin, Nobel Prize, patents and therapeutic developments

Graphical abstract Photos of Frederick Banting, John Macleod, Charles Best and James Collip around the insulin molecule (see Figures at the end)



As reported by Viktor Jörgens and Massimo Porta in the preface of their excellent book *Unveiling Diabetes - Historical Milestones in Diabetology* (**1**), two of the major advances in diabetes research to date were the discovery of the key role of the pancreas in diabetes by Oskar Minkowski and Josef von Mering in 1889 in Strasbourg and the purification of insulin and the early initiation of its clinical use by Frederick Grant Banting, Charles Herbert Best, James Bertram Collip and John James Rickard Macleod in Toronto in 1921 to 1922.

In this review commemorating the 100th anniversary of the discovery of insulin, we focus on historical aspects and attempt to show how this outstanding discovery changed the direction of diabetes research and clinical care, although milestones in basic research were attained at a slower pace.

The premises

Although the description of diabetes symptoms can be found in ancient manuscripts from Egypt, China and India, there is no doubt that in all civilisations the discomfort of diabetic patients was observed in the population. A raging thirst associated with an increased urination, excessive hunger and a sweet taste of urine could not possibly have been overlooked by the eminent physicians of the ancient times. In 2019, the International Diabetes Federation reported that 463 million people in the world (one person in 11) had diabetes (**2**).

The oldest known manuscript in which the symptoms of diabetes were mentioned is a papyrus dating from 1550 BC found in a sarcophagus in Luxor, sold to a German professor of archaeology, George Moritz Ebers in 1872 (**3**). It is now housed in the Leipzig University library. It describes various diseases and possible treatments and refers to prescriptions for

excessive urination. The Charaka's and Susruta's medical books of Hindu Medicine (1000 - 500 BC) report "a sweet taste in urines" in some disorders of urination. In the Chinese medicine, it is also reported among the signs of "Xiao-Ke" or "thirst infusing into urine", a sweet urine that attracts dogs (500 BC) (**3**). Until the 18^{h} century, attraction by sweet urine of ants, flies and bees was a biological test to detect the presence of sugar in urine and blood.

The term diabetes comes from the Greek physician Demetrius of Apamea (3rdcentury BC); it refers to a large amount of water passing through the body (**3**). It is also from the Greek Rufus of Ephesus (late 1st – early 2nd centuries AD) that the word "pancreas" was given in relation to the fleshy structure of the organ (**3**). The first accurate description of the symptoms of diabetes was made by Aretaeus Cappadocis (81-138 AD) (**3**). Avicenna, a famous physician in Persia (980-1037 AD) in his important medical book, the *Canon*, also described in detail the symptoms of diabetic patients (excessive thirst and frequent urination) associated with kidney dysfunction (**3**).

As a result, during the Middle Ages and until the 17th century, diabetes was mainly seen as a disease of the kidney and bladder.

Thomas Willis (1621-1675), the physician of King Charles I of England, and an eminent neuropathologist, is probably the first who made a distinction between diabetes "mellitus" in relation to the sweet taste of the urine, and other forms of diabetes (**4**). In 1776, Matthew Dobson (1732-1784), an English physician and experimental physiologist, showed that the sweet taste was due to the presence of sugars capable of fermentation (**5**). He also found that not only urine but blood contained sugar. John Rollo (1750-1809), a Scottish military surgeon in the Royal Artillery (**6**), in his Notes at the end of the 18th century, suggested the

first diet by restriction of carbohydrates to diabetic patients. He thought that diabetes was an affliction of the stomach (**7**).

The main breakthrough: the 19th century

In 1815, the French chemist Michel-Eugène Chevreul (1786-1889) identified the sugar in the urine as glucose (**8**). In 1848, the German pharmacist and chemist Hermann Christian von Fehling (1811-1885), developed a method for measuring glucose, which was used almost until the present day (**9**), using a solution of copper sulfate mixed with alkali and potassium sodium tartrate (Rochelle salt), known as Fehling's solution.

In the middle of the 19th century, a great step forward in our understanding of sugar metabolism was made by the French physiologist Claude Bernard (1813-1878) (**10,11,12**) (**Fig.1**). Among other important discoveries such as thermoregulation, effects of poisons such as curare in the neuromuscular junction, and the physiology of the blood gases, Claude Bernard was fascinated by the controversies between French scientists on the origin of sugar in the blood. In 1817, Bernard's mentor, François Magendie (1783-1855), in his textbook of physiology, wrote that "*it was impossible to say what is the role of the liquid of the pancreas*" (**13**). Bernard noticed that the effect of the pancreas on digestion begins just before birth and the pancreatic juice may cut fat into fatty acids and glycerol. However, in spite of his skilful expertise in vivisection, Claude Bernard was unable to carry out successful pancreatectomy in dogs (**14**). He therefore decided to focus on metabolism. In 1848, the year he co-created the Société de Biologie - where he published all his major contributions to physiology -, he showed, in the famous experiment of the "washed liver", that this organ

was able to store and produce glucose, independently of the exogenous nutrition with sugar or carbohydrates. He gave the name of glycogen to the reserve form of glucose produced by the liver in 1855 (**14,15,16**). He correctly postulated that diabetes was characterized by an overproduction of glucose by the liver (**16**)**.** Another outstanding contribution of Claude Bernard in relation to diabetes was the "diabetic piqûre" made in 1849 (**17,18**). He punctured the fourth ventricle of rabbits and dogs and, in a few minutes, observed the presence of glycosuria, thus opening a long discussion on the possible role of the central nervous system in diabetes. He was correct when he wrote that "*glycemia stays constant in normal situation whatever the alimentary diet, glycosuria is just a symptom of diabetes, not the disease itself*". In 1865, he published his masterpiece, *Introduction à l'étude de la médecine expérimentale*, still a must-read by aspirants to biomedical research (**19**).

Working in Berlin in the laboratory of Rudolf Virchow (1821-1902), a medical student, Paul Langerhans (1847-1887) (**Fig. 1**), characterized some small "heaps of cells" in the pancreas in his 1869 medical thesis (**20**). He did not work further on the pancreas and was the first to describe the dendritic cells of the epidermis (**21**). His scientific career and his premature death from tuberculosis have been detailed in the outstanding biography by Björn M. Hausen (1940-2017) (**22**). Gustave-Edouard Laguesse (1861-1927), who became Professor of Histology in Lille, France, called these cell clusters in 1893 the islets of Langerhans, and suggested they may be the source of the substance involved in blood glucose control (**23,24**). Laguesse also coined the word endocrine, which means "internally secreting" (**25**).

The last decade of the 19th century was characterized by an increased interest in diabetes, particularly in France and in Germany. Apollinaire Bouchardat (1806-1886), the leading clinical diabetologist of the 19th century, observed that weight reduction by decreasing

carbohydrates in obese patients improved diabetes. He introduced the use of physical training to decrease blood sugar in medical practice (**26**). Though he was one of the first scientists to suggest that the pancreas is the main organ involved in diabetes, it was only in 1889 that two German medical researchers, Oskar Minkowski (1858-1931) and Josef von Mering (1849-1908) (**Fig. 1**), working in the laboratory of Bernhard Naunyn (1839-1925) in Strasbourg, made one of the most important discoveries in diabetes research (**27,28**). They showed that removing the pancreas of a dog (performed to explore the role of pancreatic juices in lipid metabolism) unexpectedly induced incessant urination. Minkowski found sugar present in the urine. He and von Mering concluded that the pancreas plays a key role in diabetes by secreting a substance which lowers the level of blood glucose (**27, 28**). Whereas Claude Bernard said that pancreatectomy was impossible to be carried out in dogs, they successfully removed the gland and showed that it induced polyuria and glycosuria. More importantly, they grafted a piece of pancreas in a pancreatectomized animal and observed that the symptoms disappeared. The opposite experiment led to a definite conclusion: when the engrafted pancreas fragment was removed, diabetes reappeared (**28**).

Since then, the number of publications reporting on the putative internal pancreatic secretion that ameliorated glycosuria and hyperglycemia increased dramatically. In 1893, the French physiologist Emmanuel Hedon (1863-1933) and Gaston Giraud (1888-1975) reproduced the experiments of Minkowski and von Mering, furthermore showing that leaving a small piece of the pancreas prevented diabetes, refuting the notion that the loss of exocrine function may be the cause of diabetes (**29**). However, extraction of the internal secretion from the islets of Langerhans was challenging since they represent only a small portion of the pancreatic tissue which is otherwise mainly involved in exocrine function.

In 1909, the Belgian physiologist Jean De Meyer (1878-1934) (**Fig. 2**), at the Free University of Brussels, investigated the actions of the internal secretion of the pancreas on the kidney, and proposed to name this internal secretion "insuline" **(30**). He also demonstrated that perfusion of pancreatic extract in the liver of a diabetic dog resulted in glycogen formation (**31**)**.**

In 1916, the English physiologist Sir Edward Albert Schäfer (1850-1935) (**Fig. 2**) proposed in a compendium on *The Endocrine organs* (**32**), to name the internal secretion of the pancreas "insuline" (with French spelling), apparently unaware of De Meyer's earlier proposal. Remarkably, Schäfer also proposed in the same paper the existence of the precursor proinsuline, half a century before its discovery by Donald F. Steiner (1930-2014) at the University of Chicago (**33**). Schäfer also introduced the term endocrine, for which he is usually credited, without mention of Laguesse's proposal 25 years earlier. In 1918, he added to his last name the name of his mentor, the eminent physiologist William Sharpey (1802-1880), becoming known as Sir Edward Sharpey-Schafer.

Several researchers at the end of the 19th century and beginning of the 20th came very close to obtain a pancreatic extract able to improve glycemia (**34-36**, and references therein**)**.

The first to claim success in decreasing glycosuria in pancreatectomized dogs with a pancreatic extract was the French physiologist and endocrinologist Marcel Eugène Emile Gley (1857-1930). In 1891, he described a procedure for pancreatectomy, showing it caused experimental diabetes (**37**). In 1892, he confirmed that the atrophy of the exocrine pancreas does not cause diabetes (**38**). From 1892 to 1905, he tried to make pancreatic extracts from sclerosed remains of animal pancreas and obtained extracts that effectively diminished sugar in the urine of pancreatectomized dogs and improved their symptoms, in essence

preceding Banting and Best's result by about 25 years (**39**). Bizarrely, Gley chose not to publish his results, but instead consigned them in a sealed envelope that he deposited in 1905 at the Société de Biologie in Paris with instructions for it to be opened only upon his explicit request (**39**). He then stopped working on pancreatic extracts, claiming insufficient resources. In 1922, after Banting and Best went public with their discovery, Gley requested at the December 23, 1922 meeting of the Société de Biologie that the envelope be opened and read (**39**). He described what he carried out on February 20, 1905 with pancreatic extracts on sclerosed dog pancreas, and suggested that: "*It will be quite important to isolate the active principle of these extracts; it means the internal secretion of the pancreas and to study the mechanism of action*". Gley, unlike other protagonists in the insulin discovery saga (see below), did not attempt to claim priority, and congratulated Macleod for having achieved a great simplification of his method (**39**). Macleod acknowledged Gley's contribution in his book on *Carbohydrate Metabolism and Insulin* (**40**).

Georg Ludwig Zuelzer (1870-1949) (German spelling: Zülzer) in Berlin conducted several experiments and extraction procedures to obtain potent canine pancreatic extracts to treat diabetes (**41**). He first injected adrenal extract into rabbits and observed an increase in glycosuria. When he injected a pancreatic extract at the same time the increase in glycosuria did not appear (**42**). He filed a patent in 1912, naming the active substance acomatol (**43**), but he made a mistake by asserting that the substance was not a protein. He started to use the appropriate chemical extraction by alcohol, made a collaboration with Hoffmann La Roche, but the preparation was not pure enough to avoid serious side effects in animals and patients (**41**). The First World War unfortunately put an end to his efforts toobtain insulin. Zuelzer fled from Nazi Germany in 1934 and established a successful internal medicine practice in New York City. He died in 1949.

In 1909, Ernest Lyman Scott (1877-1966), a student studying physiology at the University of Chicago Medical School, submitted a master's thesis project to his supervisor, Prof. Anton Julius Carlson (1875-1956), focused on the search for a substance in the pancreas that lowers blood glucose (**44**). As this topic was outside of his field of research, Carlson was not enthusiastic but he agreed for Scott to carry out some experiments. In contrast to Zuelzer, and to the general belief at the time, Scott embarked on a search for a protein using an 85% alcohol extraction. In a publication in 1912 in the *American Journal of Physiology*, he described carefully an appropriate method of extraction (**44**). An anecdote is that the paper of Scott was written by Carlson without any exchange with Scott, even if the latter was the single author. In 1914 Scott published a standard test for blood glucose (**45**)**.** He became Professor of Physiology at Columbia University where he did a distinguished career having stopped working on insulin. After the publication of the Toronto research, Scott in 1923 in a correspondence to the *Journal of the American Medical Association* (JAMA) discussed his priority of the extraction procedure with a high percent of alcohol (**46**).

Nicolas Constantin Paulesco (Romanian spelling: Nicolae Paulescu) (1869-1931), a student from Romania, came to Paris to pursue his medical studies in 1888 (**41**). After receiving his MD in 1897, he worked with famous French physician and diabetologist Etienne Lancereaux (1829-1910). Lancereaux was one of the first to recognize in 1877 the likely pancreatic origin of diabetes, and he distinguished two clinical forms of diabetes, lean diabetes and fat diabetes (**47,48**). The physiologist Albert Dastre (1844-1917), a former student of Claude Bernard, suggested that Paulesco work on the isolation of the substance involved in the internal secretion of the pancreas. Paulesco returned to Bucharest in 1900 and became Professor of Physiology in 1905. Lancereaux and Paulesco published together in 1912 a monumental medical textbook (**49**). In it they argued that the internal secretion of the

pancreas affects not only the metabolism of carbohydrates but also lipids and proteins. Paulesco conducted a series of critical experiments on pancreatectomized dogs to isolate the internal secretion of the pancreas in an aqueous extract at the end of 1916, before his lab had to close because of the occupation of Romania by Germany in World War 1. This also delayed the publication of his results. He published a comprehensive Textbook of Medical Physiology (**50**). In this textbook he described his yet unpublished experiments showing that intravenous injection of his pancreatic extract induced the disappearance of diabetes symptoms in pancreatectomized dogs.

Paulesco's early results were finally published in French in 1921 in a series of short articles in the *Comptes Rendus des Séances de la Société de Biologie* (**51-54**) and in more extensive articles in the *Archives Internationales de Physiologie* (**55,56**). They were convincing and similar to what the group of Toronto published in February 1922 (see below). Paulesco named his extract "pancreine" and obtained a Romanian patent for it on April 12, 1922. Unfortunately, similarly to the work carried out by Zuelzer and others, his substance was not pure enough to avoid toxic side effects and was not administrable to humans. Paulesco attempted further purification and tried rectal administration in humans but was not successful**.** Paulesco was a fervent Christian nationalist and became involved in Romanian extreme right politics, as will be discussed later.

The biochemist Israel Simon Kleiner (1885-1966) did numerous experiments between 1915 and 1919 (**57**) while assistant in physiology at the Rockefeller Institute, demonstrating that intravenous injections of pancreatic emulsions in pancreatectomized diabetic dogs corrected hyperglycemia. There were no toxic effects if the extract was given slowly and highly diluted, suggesting a possible therapeutic application in humans. The experiments were well

controlled and carefully described, making Kleiner probably the closest of the early investigators to a therapeutic solution (**41**). The Toronto team confirmed Kleiner's experiments (**58**). Unfortunately he stopped working on this problem when he left Rockefeller in 1919.

The famed nutritionist John Raymond Murlin (1874-1960) (**59**) with Benjamin Kramer at the laboratory of Physiology at Cornell University described in 1913 pancreatic extracts that were effective in decreasing both glycosuria and hyperglycemia in pancreatectomized dogs (**60**). After a long gap in investigating pancreatic extracts, in 1922, Murlin, then Professor of Physiology at Rochester University, did some trials on diabetic patients with initial failure due to toxic side effects but later claimed some success (**61**). In 1923, he discovered glucagon (**62**).

The Toronto story

Nobody could have predicted that the discovery of insulin would take place at the University of Toronto. It was carried out by Frederick Grant Banting, Charles Herbert Best, John James Rickard Macleod and James Bertram Collip (see graphical abstract). Surprisingly enough, except for Macleod as will be discussed later, none of them had any training in endocrinology.

Frederick Banting (1891-1941)

Banting's biography has been vividly written by Michael Bliss (**63**). Frederick Banting was born on November 14, 1891 and grew up on a farm in Alliston, Ontario, a small village 90 kms northwest of Toronto. In 1912, he started medical school. His classmates included

Joseph Gilchrist and Frederick Hipwell from Alliston who played key roles in the discovery period. He set about training in the Canadian army medical service in 1915 and graduated in December 1916. As a lieutenant, he sailed from Halifax to Britain in March 1917. He was assigned to the Granville hospital at Ramsgate, in the department of orthopaedics. He stayed there for 13 months. In June 1918, he was transferred to the front, to a Canadian hospital near Cambrai in the north of France. In September 1918, as he was trying to rescue some soldiers, he was severely wounded in his right arm by a piece of shrapnel and barely escaped amputation. He stayed in Great Britain to recover until the spring of 1919, when he sailed back to Canada. He was awarded the military cross. He completed his speciality in orthopaedic surgery at the Hospital for Sick Children in Toronto but without any salary. He tried to obtain a position at this hospital but his application was declined. In the beginning of the summer 1920, he moved to London, Ontario, a small city where the University of Western Ontario is located, and opened a general practice. It was a convenient position as his fiancée was working as a teacher in a nearby town. He only saw a few patients until the fall when he supplemented his general practice with some orthopaedic cases. He felt depressed; he found a part-time job as a demonstrator in surgery and anatomy at the University. On November 1, 1920, he had to give a course on carbohydrate metabolism. He had worked all day the day before, reading all he could find about the anatomy of the pancreas and its function. He went to bed with the latest issue of the journal *Surgery, Gynecology and Obstetrics* in which he read a paper (**64**) from Moses Barron (1884-1974) (**Fig. 3**), a pathologist who was Clinical Professor of Medicine in the Department of Pathology at the University of Minnesota in Minneapolis. The article was a survey of the literature on the histology of the pancreas, the ligation of the pancreatic Wirsung's ducts, the effects of the excision of the pancreas in animals. Barron also provided figures showing lesions of

pancreas in diabetic patients suffering of obstruction of the pancreatic ducts by stones, and thus recalled in his paper the gradual atrophy of acini in contrast to the islets of Langerhans after duct blockade, as previously shown by several investigators in various animal species (**64**). Barron also reported cases in man and results by Opie and Carlson (**65,66**). He concluded that "*… the islets secrete a hormone directly into the lymph or blood streams (internal secretion), which has a controlling power over carbohydrate metabolism and which is necessary for the utilization of sugar by the tissues*". Banting was fascinated by that paper (**67**), woke up with an idea at 2:00 am on October 31, 1920, and wrote a few words in his notebook : "*Diabetus: ligate pancreatic ducts of dogs. Keep dogs alive till acini degenerate leaving islets. Try to isolate the internal secretion of these to relieve glycosurea*". In February 1923, Barron wrote a letter to Banting (**Fig. 3**) saying that "*he was flattered that Banting recalled his paper published several years before: In this article, I did not have the faintest idea or hope that it would at any time or in any way be sufficiently suggestive to start such an epoch-making investigation as you have undertaken. I feel it an honor to be in any way mentioned in connection with this work of yours and I wish that I had actually had some real part in the investigation*". In 1964, Barron received the Banting Medal of the American Diabetes Association for his contribution to the discovery of insulin.

The next morning Banting outlined his idea to Prof. Frederick R. Miller, who was in charge of the department of Physiology at London's Western University and asked permission to work on it in his laboratory. Banting thought that the reason for the failure to produce effective pancreatic extract was due to a destructive effect of trypsin on the hypothetical hormone. It would be possibly successful if the cells producing trypsin were destroyed by ligation of the duct of the gland and the remaining part of the gland could then be used as the original

material. It had previously been observed in some works, as mentioned above, that the ligation of the duct induced the atrophy of the acini but not of the islets.

Miller did not have either facilities or competence in that field and suggested Banting consult a specialist of carbohydrate physiology, Prof. John Macleod, at the University of Toronto.

John Macleod (1876 – 1935)

Macleod was born in Aberdeen, Scotland, in 1876. He graduated with a PhD in medicine in 1898. He moved to Leipzig and worked in the same laboratory where Paul Langerhans had spent a few weeks, years before. In 1903, he obtained a permanent position at Western Reserve University in Cleveland, Ohio. He developed an interest in carbohydrates, determination of blood glucose and liver glycogen and in experimental diabetes in dogs. He gained an international reputation on metabolism of various substances in particular in the field of carbohydrate metabolism with several publications and a textbook (**68**) and was appointed in 1918 to the Chair of Physiology at the University of Toronto.

On November 8, 1920, Banting had an appointment with John Macleod. Banting outlined his idea but Macleod soon realized that Banting had no real knowledge of the pancreas or diabetes and that he did not know how to carry out the appropriate experiments in order to confirm his hypotheses.

Macleod also thought that it would be risky to venture in a field in which several eminent researchers had failed. But in spite of his lack of enthusiasm, he was curious and only warned Banting that success was unlikely. Macleod respected Banting's skills and experience as a surgeon, which could be an asset to managing surgical procedures on dogs. Macleod

accepted to take Banting for a summer project, to provide a summer undergraduate student to help him and some dogs to start with. Two students were actually ready to accept the summer job, Clark Noble and Charles Best. The story says that they decided to split the job and tossed a coin to see who should start first. Best won the toss.

Charles Best (1899 – 1978)

Charles Best was born in 1899 in a small town, Pembroke, in Maine, thirty miles from the Canadian border. His father was the local physician. He went through both public and high school in Pembroke. When time came for him to decide what he wanted to do, he chose to become a physician like his father. He opted for the University of Toronto. The same year, 1917, as he entered the undergraduate BA physiology and biochemistry course at the University of Toronto, one of his aunts died of diabetes. She had been followed by Elliot Joslin in Boston. Her illness and death deeply influenced Best's orientation. Like Banting, Best was enrolled in the army after being rejected twice. He finally joined the 70th battery of the Horse Artillery in spring 1918. He was sent to England in October 1918. He returned home in December without having fought in France. He completed his second academic year in Arts and Medicine. Best worked with his close university friend Clark Noble. At a party, in February 1919, Best met Margaret Mahon. They spent their whole life together and she was a great support for him during the period before and following the discovery of insulin. Charles Best's son wrote a personal biography of his parents (**69**).

James Bertram Collip (1892 – 1965)

Collip was born in Belleville, Ontario, on November 20, 1892. In 1907, he took physiology and biochemistry at the University of Toronto and received a BA in 1912. He then undertook studies in biochemistry with the influential professor of physiology then chair of

biochemistry Archibald Macallum (1858 -1934), whom Collip would succeed at McGill University in 1928. Collip obtained an MA in 1913 and a PhD in 1917 at McGIll. He moved to the University of Alberta in Edmonton where he became professor of biochemistry. In the autumn of 1921, with a Rockefeller Travelling Fellowship, he moved for a one-year sabbatical leave to Toronto with his family and started working in John Macleod's lab. In December, his skills as a biochemist enabled him to greatly contribute to the project on insulin.

The discovery of Insulin

The story of the discovery of insulin has been plentifully reported over the years and the main source remains the book of Michael Bliss (**34**), first published in 1982; a special edition was published on the 25th anniversary of the first edition in 2007 and a new special edition for the 100th anniversary of insulin's discovery is just being published in 2021. Here we will focus on the important steps which led to the discovery, highlighting the actual breakthrough made by the Toronto team, in particular a description of the biochemical advances made to increase the purity and yield of the internal secretion of the pancreas, making insulin available to be safely used in humans.

First experiments and discovery

On May 17, 1921, just after completing his last exam for graduation from the physiology and biochemistry courses, Charles Best started working with Banting. They had discussions about the literature. With the help of Macleod, Banting began to operate on several dogs in order to ligate the ducts to obtain gradual atrophy of the pancreas as previously described (**29,64**)

or tried complete pancreatectomy (**27,28**). In mid-June, before leaving for Scotland for three months, Macleod gave Banting and Best some advice about the experiments to carry out. Banting was mainly interested in the surgical part and Best only had Macleod's courses on carbohydrate metabolism to refer to. The first results were erratic. The main problem was the summer heat that particular year in Toronto. It was very difficult to keep diabetic animals alive for a long period of time due to infections in bad sanitary animal facility conditions. A lot of dogs died even if Banting's surgical technique improved. Best was in charge of testing sugar in urine (Lewis-Benedict method) and blood (Myers-Bailey method) and to prepare some pancreatic extracts. The first encouraging data were obtained on July 30 when the diabetic dog n°410, depancreatized in two stages, showed a lower blood sugar level from 0.20 to 0.11-0.12 % (normal range 0.085-0.15 %) with intravenous injections of a pancreatic extract obtained from a duct-ligated dog degenerated pancreas sliced and chilled in Ringer solution, ground up and filtered. The other idea to graft pancreas in diabetic dogs was thus abandoned. They named "isletin" the extract prepared from degenerated pancreas and kept frozen. In late April/early May 1922 in the publication in the *American Journal of Physiology* (**70**) they settled with Macleod on "insulin", unaware of the previous naming by De Meyer and Schäfer (**30,32**). On Best's suggestion in the beginning of August 1921, Banting stopped the tedious Hedon method of two-step pancreatectomy (**29**) and it was a success, even if the dog n°408 died after four days from infection. It is the dog we can see on the famous photograph of Banting and Best on the roof of the Medical building and not, as often wrongly said, Marjorie. Best noted that the extract he obtained "*can be kept active for at least four days, is destroyed by boiling, and extracts of other organs are inactive*". He also quoted that the extracts were potent on cats and rabbits showing that it worked across species. On August 9, Banting and Best wrote a letter to Macleod reporting their data and

they mentioned the problem of infection. On August 23, Macleod answered that "*if you can prove that such extracts may reduce blood sugar and have controls, you can respond to potential criticisms. Your results are definitively positive but not absolutely certain. I will do all in my power to help you*". Macleod also gave advice to Best to carry on with the experiments.

In September, when Macleod came back to Toronto, he found that they had made the experiments he had required but he made some criticisms. He did not give any financial support to Banting. Banting was furious and wanted to leave. But the Head of the Department of Pharmacology, Velyien Henderson and Clarence Starr, the chief surgeon at the Hospital for Sick Children with whom Banting had worked in England and who had followed the work during the summer, convinced Macleod to give them more space. The first presentation of the data was given at a Journal Club on November 14, 1921.

A suggestion made after the Journal Club presentation led to the idea to carry out longevity experiments in order to see how long a diabetic dog can survive with the extracts. But they were out of duct-ligated dog extracts. Knowing that the islets developed early in pancreas development and that the external secretion is not needed until digestion begins after birth, Banting thought that foetal pancreas could be used as a source of internal secretion. They went to the local slaughterhouse to obtain some foetal calf pancreas and it worked. It was the first important breakthrough for the future experiments which confirmed that the internal secretion worked across species. Furthermore, Best changed the extraction procedure by using alcohol instead of a Ringer solution on Macleod's advice and observed that extraction of whole dog pancreas with alcohol also worked. Thus, no more degenerated pancreas was needed and adult tissue extracts could be used.

Best worked hard to improve the method of purification with 0.2% hydrochloric acid in 60% ethyl ethanol. The alcohol was evaporated and the dry residue redissolved in saline. In previously pancreatectomized dog n°35, they confirmed that an extract of its own pancreas showed a blood sugar drop from 0.38 to 0.18% in four hours. The following day the same dog received an extract of whole beef pancreas and also showed a decrease in blood sugar level from 0.28 to 0.11% in four hours (**34,71**).

On November 18, the dog n°33, named Marjorie, had her pancreas removed. She was first treated with 10cc of filtered foetal calf extracts intravenously and later with the same acid-alcohol extract but of beef pancreas, easier to obtain from the abattoir and less expensive than calf pancreas (**58**). Her urine was sugar-free after one hour and she was kept alive for 70 days, contrarily to the dogs that had not received the extract. With the help of a pathologist they obtained the proof that no pancreatic tissue remained in Marjorie to explain the impressive effects, overcoming an objection from Macleod.

In November 1921, when James Collip arrived in Toronto to work with Macleod in a lab across the street from where Banting and Best worked, he asked Banting if he needed help for the pancreatic extracts. Banting invited Collip to help estimate the glycogen content in the liver. Collip joined the team to work on the biochemistry of pancreatic extracts. With the help of Clark Noble, the extracts could be tested on normal rabbits, given an easy assay for their efficacy (**72**). Collip also developed a new blood sugar test, the Shaffer-Hartman method, based on the reduction of copper by the reducing group of the sugar, and observed that subcutaneous injections of pancreatic extracts seemed to work.

Best had trouble obtaining a pure extract with a mixture of alcohol and acid. Collip, instead of evaporating the alcohol in a warm air current as Best did, used a vacuum. He reduced the

pancreas alcoholic solution to obtain a suspension of fine particles which was filtered in order to obtain a liquid filtrate and a residue of solid particles (**58,72,73**). Collip found that a solution of the solid particles gave a potent hypoglycaemic effect with a decrease of ketone bodies and a huge increase in glycogen in the liver, suggesting that the extract could restore the function which was lacking in diabetes.

After a meeting with a group of physicians at the Toronto General Hospital, Dr Joe Gilchrist, a friend from Alliston and classmate of Banting, who was diabetic and had been on the Allen starvation diet (**74,75**) offered to help Banting as a test person. It was a dangerous test but Gilchrist had no alternative anyway. Banting gave him some extract orally (**34**) but it was unsuccessful as previously reported by Rennie and Fraser in 1907 (**76**). On December 30, 1921, Banting made a presentation at the American Physiological Society at Yale University in New Haven, Connecticut. Banting felt nervous, being an inexperienced and awkward speaker particularly in formal situations. The reaction to his presentation was extremely positive and several questions were raised by Frederick Allen, Anton Carlson, Elliott Joslin and George Clowes, director of research for the Eli Lilly company in Indianapolis, who wanted to know more about the extraction procedure. Perhaps because Banting was intimidated, Macleod felt the need, as his supervisor, to step in and support him, answering several of the questions using "we" as if he had been directly involved in the work. Banting felt that Macleod had taken the credit for the work he considered to be his and Best's work, which upset him. As for Best, he remained silent in respect for his professor (**69**).

Purification and clinical tests

At the end of December 1921, the main goal was to produce a preparation suitable for human administration. Banting insisted that the first clinical trial be made with an extract

prepared by himself and Best. Best made a batch from beef pancreas and tested it on Marjorie. It was a filtered alcoholic solution evaporated off in a vacuum still, washed with toluene and the remaining watery solution sterilized on a filter (**58**).

On January 11, 1922, the same extract was administered to a 13 year-old diabetic boy, Leonard Thompson at the Toronto General Hospital in the diabetes department of Dr Walter Campbell under the supervision of Pr Duncan Graham. It produced only modest effects (25% reduction) and the injection led to an abscess at the site of injection due to impurities.

Macleod asked Collip to make extract improvements. Collip observed indeed that results were much better if not all alcohol was evaporated (**58,72**). He carried out experiments to determine the alcohol concentration in which the active substance can be soluble in contrast to contaminants, and then increased the alcohol concentration to 90% in which the principle was able to precipitate. Collip obtained an extract purer than that of Best. He tested the preparation on rabbits and it worked, with no inflammation reported.

Thus, on January 23, two weeks after the first test, Leonard Thompson received the new extract made by Collip subcutaneously and the results were spectacular. Glycosuria and ketonuria almost disappeared, and the blood sugar dropped from 5.2 to the normal sugar concentration of 1.2 mg/cc (**77**) (**Fig. 4**). Daily injections were made until February 4th. The Connaught Laboratories (see later in another section) provided funds and lab facilities in the Medical Building to develop methods in order to increase production of the extract for clinical trials. Collip's procedure provided all the insulin used in Toronto for experimental and clinical work for the next few weeks. Collip recognized that Best was the first to demonstrate the feasibility of use of pancreatic extract. He also credited the discovery to Banting and Best. However, after a meeting with Macleod, and on the later's advice, Collip

decided not to tell the others how he had succeeded in obtaining such pure extract. The fight between them took a more dramatic turn. Macleod and Collip decided not to patent the discovery but to offer it to the University. It was proposed that a patent be taken in the names of Banting, Best and Collip on behalf of the University of Toronto. The patent was issued in 1923. In agreement with the ethical wish of Banting, the money collected from the sales would be used to set up a research fund and they would not receive any royalties. In the end of spring of 1922, Eli Lilly received the exclusive one-year licence to manufacture insulin, in partnership with the Connaught Laboratories. More details can be found later in the section: Commercialization and globalization of insulin therapy: the early days.

By February, six more patients were successfully treated and the extract was shown to produce a rise in the respiratory quotient indicating carbohydrate utilization. At the same time the presentation at the Physiological Society meeting in New Haven was published in the *American Journal of Physiology* (**78**) and the preclinical experiments in the *Journal of Laboratory and Clinical Medicine* (**71**). The clinical work was sent to the *Canadian Medical Association Journal* (**77**) **(Fig. 4)** and several papers were published in 1922 with Banting and Best alone or in association with Collip, Campbell, Fletcher, Noble and Macleod (see list in **34**).

A strange event then happened. Collip did not write down his experimental protocol, and for several weeks was unable to obtain good extracts. Nobody knows if it was due to problems with the equipment or the products used for the insulin purification, or to a change required to obtain large scale production, but no more insulin was available when the stock from Collip ended in the spring of 1922. Collip returned to the University of Alberta in early May 1922 at the end of his sabbatical year. Best took over leadership from Collip of Connaught's insulin production, completed an MA degree before entering Medical School in the fall of

1922. After several attempts, Best and his colleagues at Connaught succeeded in finding a way of providing sufficient quantities of insulin by means of a new approach using acetone with slight acidification and pH adjustments at a temperature lower than 35°C. Eli Lilly introduced a benzoic acid method of purification and a rotary high vacuum pump procedure which made possible a large scale production of insulin. Their main goal was to increase the purity and yields of insulin preparations using various chemical approaches (**58**). Banting opened his own practice in May 1922 on Bloor Street and was able to treat diabetic patients at the Christie Street Military Hospital. By late August 1922, a larger diabetes clinic was established at Toronto General Hospital and Banting was given access to its patients. John Macleod went to a Marine station in New Brunswick trying to isolate insulin from fish. The relation between "the discoverers" remained difficult: jealousy, greed, paranoia, intermingled with hard work in dreadful conditions: a good description of the situation as reported by Barbara E. Hazlett (**79**). Each of them had a personal interpretation about their respective part in the discovery.

It is under this tense situation that the Medical Research Council in Great Britain asked famous pharmacologist Sir Henry Dale (1875-1968) (who would in 1936 get the Nobel Prize in Physiology or Medicine for his work on acetylcholine), director at the National Institute of Medical Research, and a biochemist colleague Harold Dudley, to go to Toronto. They arrived in late September 1922 and were very impressed by the fact that extracts of pancreas were able to treat patients suffering from diabetes who until then had been starved to death. Dale was introduced to some of the first juvenile patients treated, Teddy Ryder and Elizabeth Hughes, the 15-year-old daughter of Charles Evans Hugues, the US Secretary of State, later Chief Justice of the Supreme Court. Another important visitor came to Toronto that autumn, at the end of November 1922: Prof. August Krogh from Copenhagen, a recent Nobel Prize

winner whose wife was diabetic. At that time, neither Banting nor Best were aware of the real significance of Krogh's visit (see below).

The press published many articles on insulin and Banting and Best received very touching letters of thanks from diabetic patients for the gift of insulin (**34,69**).

Recognition and awards (1923)

At the end of December 1922, Best and his friend Clark Noble presented a paper on the effects of insulin on rabbits at the meeting of the Federation of American Societies for Experimental Biology. Among the assistance was Robert Barany, an otologist from Uppsala, who had won the Nobel Prize in 1914 for his work on the physiology and pathology of the vestibular apparatus. He talked with John Macleod. He wrote to him in the beginning of January 1923, saying that "*the discovery of insulin is of such importance that it should be awarded the Nobel Prize*".

By February 1923, 250 physicians, 60 clinics and 1000 patients in Canada and USA were receiving insulin. Insulin was sent to many other countries during the whole year 1923.

Together with the press, politicians entered the insulin story. The Canadian Prime Minister made a recommendation to Parliament recognition for an annuity to Banting.

The story of the Nobel Prize to Banting and Macleod was written many times (**34,63,69,79**). It was the first time a Nobel Prize was awarded for such a recent discovery. Banting is still until now the youngest recipient of the Nobel Prize in Physiology or Medicine and was the first Canadian to receive a Nobel Prize. Banting was featured on the cover of Time magazine on August 27, 1923. However, it was an embarrassing choice for the Nobel Committee. In his

testimony, Alfred Nobel had not given any indication for the selection of the candidates. It only mentioned that the discovery must have an enormous impact and should be of very great practical significance. Exactly what the discovery of insulin was. The awardees had to be alive and could not be more than 3 at the same time. Unfortunately there were four of them in the Toronto team.

The Nobel committee knew about the bad relationship between the members of the Toronto team. Some members of the Committee quoted that point as an argument to postpone the prize, arguing that it was a too recent discovery. However, with the pressure from the media, from some other authorities such as Henry Dale as well as from the patients themselves, it would have been incomprehensible and in disagreement to Alfred Nobel's testimony, not to award the Nobel Prize for the discovery of insulin. As we know from the Nobel records, Krogh nominated Banting and Macleod and also suggested the name of Best. George W. Crile from Cleveland followed Krogh. George N. Stewart, also from Cleveland, nominated only Macleod. Finally Francis G. Benedict, from Washington, nominated only Banting (https://www.nobelprize.org/nomination/archive/). The Nobel Prize to Banting and Macleod was voted on October 25, 1923 by the committee of the Karolinska Institute. On October 26, news about it spread in Toronto. Banting drove back to Toronto from Alliston. When he arrived in his office, the phone was ringing. It was a friend who congratulated him and told him to open the newspaper. When he knew that Macleod had won the Prize with him, Banting's anger about Macleod's inclusion was unbelievable. At the same time, Best was giving a lecture at Harvard University. The students were very impressed at such discovery made by a young student. At the end of his talk, Dr Joslin read a telegram he had just received from Banting saying "*I share the Nobel Prize with Best*" (**Fig. 5**). Macleod did the same with Collip **(Fig. 5)**. None of them attended the official ceremony in Stockholm on

December 10. It was the first time that happened for the attribution of the Nobel Prize in Physiology or Medicine.

The aftermath of the discovery

The Nobel Committee received letters of protests from Zuelzer and Paulesco claiming priority, which were ignored. Paulesco became a kind of "cause célèbre" for the Romanian scientific (and political) community which still today considers that the Nobel was stolen from him by the Canadians. The fact that Banting misinterpreted and misquoted Paulesco's French publication did not help the Romanian's feeling that the Canadians had cheated. The controversy has been largely discussed over the years (see refs. **1,34,63,69**). There is no doubt that Paulesco was a gifted scientist and he had his supporters both within and outside Romania (**80-82**). The problem with attempts at his rehabilitation has been the increasing awareness of his militant fascist activities (**41,83,84**). Paulesco mixed his science with religion and extreme right politics. He died in 1931.

The following years for the Toronto investigators were honours, awards, receptions, lectures between work and writing papers. During a visit to Europe in 1934, Banting was knighted by King George VI of UK.

It is only in 1925 that Macleod and Banting delivered their Nobel Lecture in Stockholm (**85,86**).

At the same time, Best arrived in London with Margaret Mahon. He was to work on blood pressure with Henry Dale after completing his degree in medicine. In Dale's laboratory, Best identified two depressor substances present in liver extracts, histamine and choline (**87**). He also described an enzyme, histaminase, able to cause the disappearance of histamine from

lung tissue (**88**) and made important contributions on heparin, an anticoagulant drug (**89**). Best was nominated several times for the Nobel Prize in relation with these discoveries.

In 1928, Macleod, whose health had been deteriorating with arthritis, decided to leave Toronto and return to Aberdeen. Best was strongly supported to replace Macleod as professor and head of the department of Physiology at the University of Toronto in 1929 after a year of co-operation with Macleod on the grounds of his youth: he was only thirty years old. In September 1930, a new lab facilities building was officially opened at 100 College Street in honor of Frederick Banting, the Banting Institute. Next to it, the Best Institute was opened in 1950.

Charles Best published around 200 papers of high quality. He also set up a blood donor service which was very efficient, particularly at the beginning of World War II, when they prepared millions of dried serum and shipped them to England (**90**). The Connaught Laboratories produced large quantities of heparin for the prevention of blood clotting during transfusion.

Frederick Banting was not as successful as Best following the discovery of insulin (**34**). He mainly wrote clinical papers. He became professor of Medical Research in the department of Pharmacology. He set up his own clinic for diabetic patients with some appointments from the General Hospital. His friends from Alliston, Joe Gilchrist and Fred Hipwell, took care of the clinic at 160 Bloor St in Toronto. Banting moved to cancer research. He married his second wife Henrietta, "*Lady Banting*" in 1939. His best achievement during the years after the discovery of insulin was his work at the National Research Council of Canada (NRC). He developed medical research in Canada, its organization, pushing to establish competitive laboratories with grants and stipends for young talented Canadian researchers. He also worked on biological warfare issues. Interestingly, two of the committee's members were

Best and Collip. In November 1939, when Best wrote to Dale about linking British and Canadian war effort on research programs, Banting, who had been nominated in 1938 as head of the medical research in NRC was irritated by Best's initiative. Banting thought it was up to him to talk to Dale about common medical war problems. Banting thus decided to go to England himself and meet Dale in February 1941.

Unfortunately, he never reached England. He died in an airplane crash on February 21, 1941 at the age of 50. His death in a Hudson bomber over Newfoundland remains an enigma. Was it sabotage or a pilot error due to bad weather or a technical problem? (**91**). Banting had official funerals.

John Macleod had returned to Scotland in 1928. He had a position as professor of physiology at the University of Aberdeen. He then worked on the observation of Claude Bernard on the hyperglycemia due to puncture of the 4th ventricle. He found that gluconeogenesis in rabbit liver was controlled by parasympathetic innervation. He died in Aberdeen on March 16, 1935 at the age of 59.

When James Bertram Collip definitively returned to the University of Alberta in 1922, as a full professor and head of the department of biochemistry, he moved away from general research in the field of insulin and focused on the identification and isolation of hormones of therapeutic value. Collip was a leading researcher in endocrine science during the 1920s and 1930s. His most important contribution in the first years after the insulin discovery was, in 1924, the isolation of the parathyroid hormone (**92**). In 1928, Collip was appointed to the chair of biochemistry at McGill University, succeeding his mentor Archibald Macallum. Among his prestigious graduate students was Hans Selye, "*the father of stress*", with whom, in 1933, in collaboration with Anderson and Thompson, he purified an adrenocorticotropic

hormone (ACTH) extract (**93,94**). Collip also worked on the identification of thyroid-stimulating hormone (TSH) (**95**). In 1930, in collaboration with a small Montreal pharmaceutical firm, Ayerst, Collip isolated what he called Emmenin, a form of estriol from the placenta that was commercially significant because it could be administered orally (**96**). Later, the Ayerst researchers, on Collip's advice and using his standardization procedures, developed a different orally active estrogen from pregnant mares' urine called Premarin. Used in hormone replacement therapy for the treatment of menopausal symptoms, it became one of the top-selling pharmaceutical products in the second half of the 20th century.

Between 1938 and 1957, Collip had a leading role in establishing federal coordination and funding of medical research through the NRC. In the mid-1930s, he reconciled with Banting, and when Banting organized the Associate Committee on Medical Research for the NRC in 1938, he asked Collip to join. After Banting's death, Collip assumed leadership of the medical-research arms of the NRC and served as Canada's medical liaison officer to Washington (**97**). In 1946, he became the first director of the Division of Medical Research (**98**). Because of Canada's limited population, Collip argued against building national laboratories but instead promoted grants-in-aid for research at universities and teaching hospitals. In 1947, he became dean of medicine at the University of Western Ontario (**99**). Collip received many honors during his career. He died of a stroke in 1965 at the age of 72. He found the bitter conflict associated with the discovery of insulin absolutely distasteful. Known for his integrity, modesty, and quiet nature, Collip always refused to discuss the insulin days, only saying that "*he was sure the historical record would speak for itself"* as reported by Alison Li in her excellent book on Collip (**98**).

Fred Banting, Charley Best, John Macleod and Bert Collip, as they were called, are all four as well as historian Michael Bliss inductees into the Canadian Medical Hall of Fame in London, Ontario. (see more on the Defining Moments Canada "Insulin 100" website https://definingmomentscanada.ca/insulin100/).

Commercialization and globalization of insulin therapy: the early days

The early developments of the pharmaceutical production of insulin have been discussed in several books and reviews (**34,36,100,101**). Later and contemporary developments in insulin therapy have been thoroughly reviewed recently in this journal by Irl Hirsch and colleagues (**102**). In this review, we will briefly summarize here the early developments.

Patent issues

Initially, the Toronto team started the production of insulin in collaboration with the Connaught Anti-Toxin Laboratories, founded in 1914 by a professor of hygiene, John G. FitzGerald (1883-1940) (**34**) (see below). The agreement, dated January 25, 1922, precluded that Banting, Best and Collip get into any patenting agreement with any commercial firm. Eli Lilly had already expressed interest (see below). Faced with difficulties in large scale production, the Toronto team and FitzGerald decided on May 22, 1922 to accept the offer of collaboration from Eli Lilly and Co, and to apply for an American patent. Lilly would retain the territorial rights to the United States, Central and South America. The US patent application ran into initial trouble when the chief American patent examiner became aware of the 1912 US patent granted to George Zuelzer. The patent was also contested by John R. Murlin and reluctantly by Ernest L. Scott (**34**). After support from a number of eminent scientists and also Charles Evans Hughes, the patent was finally granted under the condition

that Banting was added to the inventors to avoid further challenge on the basis of Collip and Best not being the sole inventors. Banting's name was also added to the British and Canadian patents. The three men promptly assigned their patent rights to the Board of Governors of the University of Toronto in exchange of one dollar each.

The major players

The Connaught Anti-Toxin Laboratories in Toronto

As described by Michael Bliss (**34**), the Connaught Laboratories played a critical role in the early production and distribution of insulin in Canada and abroad. Yet, as pointed out by Bliss's history student Christopher J. Rutty (**103**), the book provides only limited detail about the Laboratories and their history, because at the time Bliss did his research for the book published in 1982, the Connaught's archives had not been collected and catalogued. A 1968 book written by Connaught's second director Robert D. Defries on the first 40 years of the laboratories is cited in Bliss's book (**104**).

The Connaught laboratories were the brainchild of an entrepreneurial, restless, enthusiastic and ambitious young physician, John Gerald FitzGerald, born on December 1882 in a rural Ontario village (**103**). He suffered from a manic-depressive condition and committed suicide in 1940. Yet, his vision and determination completely changed the landscape of Public Health in Canada as well as the role of the University of Toronto's research in fighting major diseases.

The Connaught labs, besides their role in the insulin saga, made major contributions to the research development and large-scale production of a large range of biological products, including diphteria toxoid, pertussis vaccine, heparin, penicillin, combined vaccines, the Salk and Sabin polio vaccine, and smallpox vaccine (**103,104**).

In early 1911, FitzGerald established a Laboratory of serum diagnosis in the Department of Pathology and Bacteriology at the University of Toronto. In the fall of 1913, he built a small stable and laboratory behind a private house in West Toronto hosting four horses, which enabled him to produce large doses of diphteria antitoxin for the Provincial Board of Health. In 1914 FitzGerald persuaded the University of Toronto to assume responsibility for his antitoxin production. The Antitoxin Laboratory was established in the Department of Hygiene on May 1st, 1914, in the basement of the Medical Building. It produced tetanus antitoxin for the Canadian troops fighting in Europe. To support the war effort, Colonel Albert E. Gooderham, a local distiller and member of the university board of directors, as well as chairman of the Ontario Red Cross Society, funded the purchase of farmland 12 miles north of Toronto in York township, and the erection of new buildings. The facility was handed to the University of Toronto on October 25, 1917. It was named "Connaught Antitoxin Laboratories and University Farm", after the Duke of Connaught, patron of the Canadian Public Health Association and a friend of Gooderham. In 1946 it became "Connaught Medical Research Laboratories". It remained a self-sustaining part of the University of Toronto until 1972, when the University sold it to the Canadian Development Corporation, owned by the Canadian Government. In 1989, the Connaught labs through a variety of mergers became part of Sanofi Aventis. Its vaccine business became Sanofi Pasteur, of which the former Connaught is the Canadian component.

Connaught produced insulin until 1983. From 1984 until 1993Connaught supplied Canada with insulin from Novo, later Novo Nordisk after the 1989 merger, to whom it had leased its production facility.

Charles Best became director of the Connaught Laboratories Insulin Division from 1922 to 1925, then Assistant Director from 1925 to 1931 and Associate Director from 1931 to 1941. He led the development of the anti - coagulant heparin (**89**).

David Aylmer Scott (1892-1971), who was part of the heparin team, also played a major role in standardizing the conditions for successful crystallization of insulin (see below).

A detailed history of the Connaught Laboratories written by Christopher Rutty (**103**) as well as a rich iconography can be found on the Web Site of the Connaught Fund: Connaught.research.utoronto.ca/history.

Eli Lilly and Company in Indianapolis

Eli Lilly and Company, in Indianapolis, Indiana, USA, was founded in 1876 by, and named after, Colonel Eli Lilly (1838-1898), a pharmaceutical chemist and veteran of the American Civil War (on the Union side) (**105**). It started as a small, two-story building near the main business street of Indianapolis. In 1890, Colonel Lilly turned over the management of the company to his son Joshuah Kirby Lilly Sr (1861-1948), who ran the company for 34 years and oversaw a major development. A critical step was when Joshuah Lilly hired British-born and trained biochemist George Henry Alexander Clowes (1877-1958) in the summer of 1919 as Director of Biochemical Research. Clowes transformed Eli Lilly and Company into a major science-based pharmaceutical company, and put a lot of emphasis in fostering collaboration with external academic scientists (**106,107**). A fateful encounter happened when Clowes attended the presentation by Macleod, Banting and s Best at the December 30, 1921 session of the American Physiological Society conference at Yale University in New Haven. Clowes in the end managed, as narrated above, to secure the rights for Eli Lilly and Company to produce and sell insulin to American patients. There were some fights between Eli Lilly and Co and the Toronto researchers on the brand name for Lilly insulin. It ended up as Iletin with

insulin as a subtitle. By the end of 1923, nearly 25.000 Americans were receiving insulin. The company's profits soared. Clowes got the Banting Medal of the American Diabetes Association in 1947 for his role in making insulin widely available. A lively biography of Clowes has been written by his grandson (**107**). The initial insulin success was the first step in Eli Lilly and Co becoming the market leader in insulin and diabetes therapy for decades. They would again make history in 1982 by launching DNA recombinant human insulin under the name Humulin, the first recombinant protein therapeutic (**108-110**).

Nordisk Insulin laboratorium and Novo Therapeutisk Laboratorium in Denmark

A leading figure in setting up the production and commercialization of insulin in Europe was the Danish zoologist Shack August Steinberg Krogh (1874-1949), known as August Krogh (**100,111,112**) (**Fig. 6**). Krogh became professor at the Department of Zoophysiology at the University of Copenhagen from 1916 to 1945. His wife Marie (**Fig. 6**) was a physician and his lifetime close collaborator. A biography of August and Marie Krogh was written by their daughter Bodil Schmidt-Nielsen in 1995 (**113**).

August Krogh obtained the Nobel Prize in Physiology or Medicine in 1920 for discovering a mechanism that opens and closes blood capillaries according to the tissues need for oxygen. He received invitations to give lectures in prestigious place in both Europe and the USA. However, Krogh had to cancel his travels because his wife had fallen ill in the summer of 1920. She consulted a young physician that Krogh knew since 1919, Hans-Christian Hagedorn (1888-1971) (**Fig. 6**). He was already well-known for having designed, together with Birger Norman Jensen (1889-1946), a new and very accurate micromethod for measuring blood glucose that did not require puncturing the veins (**114**). Hagedorn had just passed his dissertation in the Faculty of Medicine. He found out that Marie had diabetes. Neither insulin nor oral hypoglycemic agents were available in 1922, so Hagedorn put her on a strict

diet and she progressively improved so that they could start travelling in September 1922 (**100**). They first went to London and met Ernest Henry Starling (who coined the word "*hormone*" in 1905), who informed them of the discovery of insulin. They also heard that Krogh's friend Sir Henry Dale had gone to Toronto to investigate (**115**). The Kroghs arrived in Boston at the end of September. They met famous diabetologist Elliott P. Joslin (1869-1962), the first American doctor to specialize in diabetes treatment, and founder of the Joslin Diabetes Center at Harvard Medical School. He was treating patients with insulin. Krogh wrote to Macleod to enquire about how to start producing insulin in Denmark and was immediately invited to Toronto. Being able to treat Marie's diabetes was obviously a strong motivation. The Kroghs stayed at Macleod's house from November 23 to 25, 1922. They also met Banting and two of his patients including Elizabeth Evans Hughes (**111**). Krogh followed the procedure used by Henry Dale to acquire the license for Britain (see below): he contacted the Insulin Committee set in place by the University of Toronto to grant licences, and obtained one to produce insulin for Scandinavia.

Krogh returned the favour by successfully nominating Banting and Macleod for the Nobel Prize. Upon return to Copenhagen in December 1922, the Kroghs immediately met with Hagedorn and set up to produce insulin from ox or calf pancreases (later they would switch to pigs) in the villa of Hagedorn and his wife Maria in Hellerup, north of Copenhagen (**111**). They quickly realized that they needed professional help in fabrication, production and distribution and made an alliance with the pharmacist August Kongsted (1870-1939), owner of Lovens Kemiske Fabrik (Leo Pharmaceutical Products). Together they founded the independent Nordisk Insulin laboratorium later known as Nordisk Gentofte A/S, headed by Hagedorn. Insulin was produced as insulin Leo. In 1932, Nordisk opened a diabetes hospital in Gentofte, named Steno Memorial Hospital, later renamed Steno Diabetes Centre, also

headed by Hagedorn. In 1957, Nordisk opened an independent research laboratory devoted to diabetes research in Gentofte, named Hagedorn Research Laboratory (later Hagedorn Research Institute). One of us (PDM) was Research Director of the Hagedorn Research Institute from 1990 to 2000.

Back in 1924, a fateful event happened in the Nordisk factory (**111**). A chemist hired by Hagedorn in October 1923, Thorvald Pedersen **(Fig. 7)**, got into a conflict with Hagedorn, who could be sometimes autocratic and temperamental. Thorvald was fired. Being unemployed, he decided to start making insulin of his own together with his older brother Harald, an engineer who worked with August Krogh since 1918 (**Fig. 7**). They founded their own firm, Novo Therapeutisk Laboratorium, (later Novo A/S), on 16 February 1925 (**111,116**). They started producing insulin in Harald's family villa in Frederiksberg, part of Copenhagen. They subsequently expanded in buildings in Copenhagen and Bagsvaerd.

Nordisk and Novo both became major players in insulin and diabetes treatment in Scandinavia, Europe and rest of the world, and competed fiercely for 64 years until they merged in 1989 into Novo Nordisk A/S – which today is the market leader in diabetes products worldwide.

Edinburgh University, the Medical Research Council and Burroughs Wellcome in Great Britain

The first successful use of insulin in Europe happened in Edinburgh thanks to a Canadian cardiologist, Jonathan Campbell Meakins (1882-1959) (**100**), who was knowledgeable about diabetes (**117**). While traveling through Canada in February 1922, he heard about the announcement about insulin. Meakins had a colleague, Prof. Norman Purves Walker (1862-1943), who had developed diabetes. Meakins wrote to Macleod on June 1922 for more information on making insulin and maybe getting some supplies. Macleod sent detailed

manufacturing instructions. Meakins sent one of his assistants to Toronto who brough back the first supply of insulin to Great Britain. However, Meakins with biochemist Charles Robert Harington (1897-1972) (best known for synthesizing thyroxine) made themselves a first batch of porcine insulin that was so potent it killed the test rabbits. They diluted it to a hopefully safe level. Norman Walker volunteered to take it. This occurred at the beginning of August 1922 at the Royal Infirmary of Edinburgh, where Walker was in charge of the dermatology department. As described by Meakins, "*the result was perfect*" (**117**). Meakins's insulin became commercially available in 1923.

In July 1922, the University of Toronto enquired whether the Medical Research Council (MRC) would be interested in acquiring the patent rights to produce insulin. The MRC, first skeptical, sent as described above Sir Henry Dale to Toronto to investigate. He came back enthusiastic. The MRC after doing some studies in local hospitals supplied the first British-made insulin to hospitals on April 12, 1923 (**100**). Eventually insulin was commercially produced by one of the largest and most successful British pharmaceutical companies, Burroughs Wellcome. It was marketed as "Wellcome insulin".

The National Health Institute in Poland

Surprisingly, Poland which was reborn at the end of WWI after 123 years of not existing as a state, managed to produce insulin at the National Health Institute and to treat diabetic patients at the Holy Spirit Hospital in Warsaw only 2 years after Toronto and one year after Denmark. This story was not well known and was recently unraveled by Dr Leszek Czupryniak, professor of Diabetology and Internal Medicine at the Medical University of Warsaw (**100**).

The mastermind behind the National Health Institute was the Warsaw-born physician and bacteriologist Ludwik Witold Rajchman (1881-1965). During WWI he worked at the MRC

where he developed his interest in infectious diseases. After the war he decided to set up a similar institution in Warsaw with the main task of producing sera and vaccines and to fight infectious diseases (like the Connaught Antitoxin Laboratories) and became the general director of the National Health Institute. He got substantial support from the Rockefeller Foundation.

In the spring of 1923 while visiting the Rockefeller Foundation, he reacquainted himself with the Warsaw-born biochemist Casimir (Kazimierz) Funk (1884-1967), working then at the Biochemical Department of Columbia University. Funk became famous in 1911-1912 for discovering and naming vitamins (**118-120**). Rajchman invited Funk to become the head biochemist at the National Health Institute, which Funk gladly accepted. He arrived in Warsaw in October 1923. Funk conceived the idea to make and sell insulin, which then was not manufactured in Poland and had to be imported at heavy cost, to fund his laboratory. He was successful. Polish production at the National Health Institute continued until the late 1950s and then moved to a larger factory in an industrial part of Warsaw.

Rajchman founded UNICEF after WWII and was his chairman of the board until 1950. Describing his remarkable biography is outside the scope of this review, for details see ref. **121**.

Hoechst A/G in Germany

Hoechst was originally a chemical company founded in 1863 by German chemists Eugen Nicolaus Lucius (1834-1903) and Carl Friedrich Wilhelm Meister (1827-1895). The later name Hoechst comes from its location in the village of Höchst am Main near Frankfurt. In 1906 it synthesized adrenaline. By the late 20th century, it had become one of the world's largest producers of pharmaceuticals and went through multiple mergers and acquisitions to become like the Connaught laboratories part of Sanofi. Hoechst began to study new ways to

treat diabetes in 1910. In 1921 they isolated from cattle pancreas an extract that reduces blood glucose. In 1923, Hoechst made insulin available to German doctors. Subsequent progresses in insulin manufacturing at Hoechst can be found in ref. **122**. Rolf Geiger (1923-1988) later became one of their best-known insulin chemists.

Crystallization of insulin and the role of zinc: John J. Abel and David A. Scott

Insulin was crystallized for the first time in 1926 by John Jacob Abel (1857-1938) at Johns Hopkins University in Baltimore (**123-127**) (**Fig. 8**). The biography of John J. Abel and his critical role in the development of pharmacology in the United States are well described in the excellent book by American historian John Parascandola (**123**). Abel developed the first Department of Pharmacology in North America in 1890 at the University of Michigan. In 1893 he moved to Johns Hopkins University where he became America's first full-time professor of Pharmacology. He stayed there until he retired in 1932. Among his achievements are the isolation in 1897 of adrenaline (which he called epinephrine) as crystals of benzoyl derivative, and the first kidney dialysis machine. He founded the Journal of Biological Chemistry in 1905 and the Journal of Pharmacology and Experimental Therapeutics in 1908.

John Abel turned his attention to insulin recently discovered in Toronto in 1924. It was recognized that the Toronto preparation contained impurities, and that it would be desirable to isolate the hormone in pure form. There was also disagreement about its chemical nature (**123**). At the end of 1925, Abel first obtained insulin crystals (**124-126**). This was a major step in obtaining a pure insulin preparation. The crystals were also used to confirm that insulin is a protein. Vincent du Vigneaud (1901-1978), an American biochemist, did his Ph.D with John R. Murlin at University of Rochester and showed in his thesis work, published in

1927 (**127**), that purified insulin contained disulfide-linked cystine. He moved to Abel's lab at Johns Hopkins in 1925 as a post-doc to work on the composition of crystalline insulin, and demonstrated the presence of amino acids cystine, tyrosine, histidine, arginine and lysine making about 30% of the molecule, and speculated that the rest of the molecule is also made of amino acids (**128-130**), strongly suggesting the proteic nature of insulin (**131**). Du Vigneaud won the Nobel Prize in Chemistry in 1955 for the synthesis of oxytocin.

Abel was 69-years-old in 1926 and a bit absent-minded (**123**), and he soon had trouble reproducing his crystallization results by his original method. After a year he succeeded again using a somewhat modified technique. He then withdrew from insulin research and left it in the hands of his colleagues.

It is only in the early 30's that it was found that the key to a stable crystallization of insulin was the presence of metal ions such as zinc. David A. Scott of the Connaught laboratories while on sabbatical in London, UK, tried to crystallize insulin using Abel's method, without success (**Fig. 8**). Back at Connaught in January 1930, Scott realized that pancreatic tissue contains appreciable amounts of zinc, cobalt and nickel. He discovered that the addition of small amounts of zinc chloride to a buffered solution of insulin resulted in the precipitation of insulin crystals. He showed that by refining this process large quantities of highly purified insulin could be consistently produced (**132,133**). This was a major breakthrough in the early history of the insulin saga since it opened the way to the development of ultra-pure insulins and insulins with different PK/PD properties (**102**), as well as paving the way to solving the three-dimensional structure of insulin (**134**), although this would take another 35 years. David A. Scott shared the Banting Medal of the American Diabetes Association in 1964 with Moses Barron and two others.

A brief glimpse of later milestones

The sudden availability of insulin from early 1922 was truly a miracle for the young patients who were slowly dying of starvation on Allen's diet (**75**), and for the parents who witnessed their resurrection. Further milestones in insulin therapy and diabetes treatment have been recently reviewed (**1,102,135,136**)**.** Insulin was also a miracle for scientists interested in basic research on proteins. Many of the major technical advances in studying proteins were made using insulin first. It was the first protein to be sequenced in 1951-1955 (**137**), earning Fred Sanger his first Nobel Prize in 1958, the first protein to be crystallized as narrated above, the first protein to be synthesized in the early 60's in the United States (**138**), Germany (**139**) and China (**140**), the first protein hormone to have its plasma level measured by radioimmunoassay in 1960 by Solomon A. Berson and Rosalyn S. Yalow (**141**), earning Yalow the 1977 Nobel Prize in Physiology or Medicine after Berson's death in 1972 (**142**), and the first protein therapeutics made from recombinant DNA to be approved by the FDA in 1982 (Humulin Lilly) (**108-110**). Since the scope of our review is limited to the periods preceding and immediately following the 1921 discovery, we will not elaborate further here on subsequent milestones (reviewed elsewhere (**36,143,144**).

Summary and conclusions

The triumph of the Toronto team in producing an efficient insulin extract that was suitable for injections in humans started as an unlikely story. Many experienced researchers had tried unsuccessfully to tackle this problem. Their preparations lowered blood glucose in pancreatectomized dogs but were too toxic for treating humans. A 29-year-old surgeon with no research experience and no expertise in diabetes or endocrinology, Frederick Banting, had an epiphany upon reading in bed a paper from a Minneapolis pathologist who described

the effect of pancreatic duct ligation on exocrine and endocrine pancreas, showing degeneration of the exocrine but not the endocrine pancreas. Banting surmised that destruction of insulin by pancreatic enzymes may be the problem in getting an active extract, and, inspired by the fact that ligation is a surgical procedure, sketched a protocol (in which he could not even spell diabetes) based on this procedure. As persistent as he was naïve, he persuaded Prof. Macleod to let him go ahead with the help of an equally untrained medical student, Charles Best. They managed, despite all odds, to get to the stage where several had been before, rescuing pancreatectomized dogs. It turned out that the duct ligation was useless, the pancreatic enzymes being in an inactive proenzyme form, and could be omitted entirely. It is when Collip became involved that significant progress in purification was achieved.

Thus, this "discovery of insulin" was not a paradigm shift as defined by Thomas Kuhn (**145**) but rather a paradigmatic incremental progress in purifying what was not even known yet to be a protein. The true paradigm shift had been Minkowski and von Mering's 1889 experiment, a serendipitous result that was a game changer. The successful purification of insulin was unavoidable given the progress already accomplished. It is likely that the motivation for the Nobel award was not just the purification of a pancreatic extract, but the demonstration that it saved the life of dying diabetic patients, which no other precursor had achieved. This is what struck August Krogh (and his diabetic wife) when they visited Toronto in 1922, leading him to nominate Banting and Macleod for the Nobel Prize. Had the Nobel Committee been wise in specifying this fact, and not just "discovery of insulin", much controversy would have been avoided.

The controversy in limiting the award to Banting and Macleod, however, would continue, as discussed in the last chapter of Michael Bliss's 25th anniversary edition of his book (**34**) and in refs. **146** and **147.** Sadly, the relationship between the members of the Toronto quatuor, already bad before the award, steadily deteriorated afterwards in large part due to Banting's paranoid dislike of Macleod. Jesse Roth and colleagues speculated that he may have suffered of posttraumatic stress disorder after his combat experience in WWI where a shrapnel wound to his right arm almost ended in amputation (**147**). Macleod went back home to Aberdeen in 1928 disenchanted by his Toronto experience and did not comment on it afterwards. Banting did not achieve any notable scientific breakthroughs until his tragic death in 1941. Best and Collip pursued distinguished academic and scientific careers.

Banting and Best spent considerable energy in the rest of their life, quite successfully, to perpetuate the myth that insulin was discovered by Banting and Best, and to demean the role of their colleagues (**34,69,146,147**). Collip maintained a dignified silence on the issue. It is the great merit of historian Michael Bliss to have carefully investigated and documented the respective contributions of the four team members, and to have rehabilitated the reputation of John Macleod in his 1982 book on "The discovery of insulin" and successive editions (**34**) and his subsequent Banting biography (**63**), as well as in many scholarly publications. As Llewellys Barker said at a tribute at the University of Toronto in November 1923: "*There is in insulin glory enough for all*". This inspired a remarkable TV film produced by Gordon Hinch on the insulin discovery entitled *Glory enough for all*, based on Michael Bliss's book, released by the Canadian Broadcasting Corporation on June 28, 1988. It won nine Gemini Awards in 1989.

The above considerations in no way diminish the merits of the Toronto team as a whole nor the miracle of being able to immediately resuscitate patients dying of diabetes. Some of

them survived the insulin discoverers by many years. The isolation and purification of insulin in Toronto in 1921 undoubtedly represents one of the major medical breakthroughs of the early 20th century.

To conclude on a sobering note, as mentioned by Banting in his Nobel lecture, "*insulin was not a cure for diabetes; it is a treatment*". Despite a century of research, we seem nowhere near being able to prevent the autoimmune aggression that kills beta cells in type 1 diabetes, or to replace them by stem cell therapy. We seem impotent in the face of the raging worldwide epidemics of type 2 diabetes and obesity. In addition, while the insulin pioneers refused to make profit from insulin sales, the current business model of insulin distribution and sales, and health care insurance, has resulted in a situation where even in some rich countries many people cannot afford insulin and have to ration their treatment (**101,148**). Some new breakthroughs are badly needed.

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Essential points

- Diabetes mellitus is an ancient disease that was fatal until insulin became available. Today there are an estimated 463 million people with diabetes worldwide.
- The discovery of a substance which regulates blood glucose levels was a long scientific process. Minkowski and von Mering in 1889 demonstrated its pancreatic origin.
- Over the following 20 years, animal pancreatic extracts that lowered blood glucose in pancreatectomized dogs were obtained by a number of investigators, but none of these extracts were suitable for human administration.
- The purification of insulin was improved in 1921 by Frederick Banting, John Macleod, Charles Best and James Collip at the University of Toronto, and the first patients were successfully treated in 1922.
- The Nobel Prize in Physiology or Medicine was awarded to Frederick Banting and John Macleod in 1923 “for the discovery of insulin”; its rapid clinical application was clearly a determining factor in the award.
- Obtaining large quantities of purified insulin, while challenging, proceeded rapidly for industrial development.
- The availability of insulin paved the way to considerable developments in basic research, but this progress was much slower than the clinical applications.

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Figure legends

Figure 1

Top left: Claude Bernard. Top right: Paul Langerhans. Bottom left: Oskar Minkowski. Bottom right: Josef von Mering. All images in public domain, used with permission.

Figure 2

Left: Jean De Meyer. From De Meyer's obituary in Bruxelles Médical 1933-1934 p. 1199, courtesy of Françoise Delloye, librarian at Archives of Free University of Brussels, used with permission of "La Revue Médicale de Bruxelles".

Right : Sir Edward Sharpey Schafer. Photograph by J. Russel and Sons. Wellcome Collection. Attribution 4.0 International (CC BY 4.0)

Figure 3

Left: Moses Barron. From Archives of University of Minnesota: https://med.umn.edu/news-events/medical-bulletin/behind-scenes-heroes, used with permission.

Middle: Barron's article that inspired Banting. Courtesy of American College of Surgeons, used with permission.

Right: Letter of Barron to Banting. https://insulin.library.utoronto.ca/islandora/object/insulin%3AL10031. Courtesy of Thomas Fisher Rare Book Library, used with permission.

Figure 4

First report of human treatment by Banting and colleagues (reference **77**).

Figure 5

Telegram from Banting and letter from Collip about Nobel Prize money being shared. Images courtesy of the Thomas Fisher Rare Book Library, used with permission.

Figure 6

Left: August and Marie Krogh. From Novo Nordisk 100 Years: https://www.novonordisk.sg/about/insulin-100-years.html . Novo Nordisk History and Art Collection, used with permission.

Right: Hans-Christian Hagedorn. From reference **111**. Novo Nordisk History and Art Collection, used with permission.

Figure 7

Thorvald (left) and Harald Pedersen. From reference **116**. Novo Nordisk History and Art Collection, used with permission.

Figure 8

Top: John Jacob Abel and his insulin crystals. Abel's picture from: https://academictree.org/chemistry/publications.php?pid=1890. Copyright The Academic Family Tree. Attribution CC-BY 3.0. Crystals picture from reference **124**.

Bottom: David A. Scott and his zinc insulin crystals. Scott's picture from https://insulin.library.utoronto.ca/islandora/object/insulin%3AP10082. Crystals picture from reference **132**. Courtesy of Sanofi Pasteur Canada (Connaught Campus Archives), used with permission.

Figure 1

Figure 2

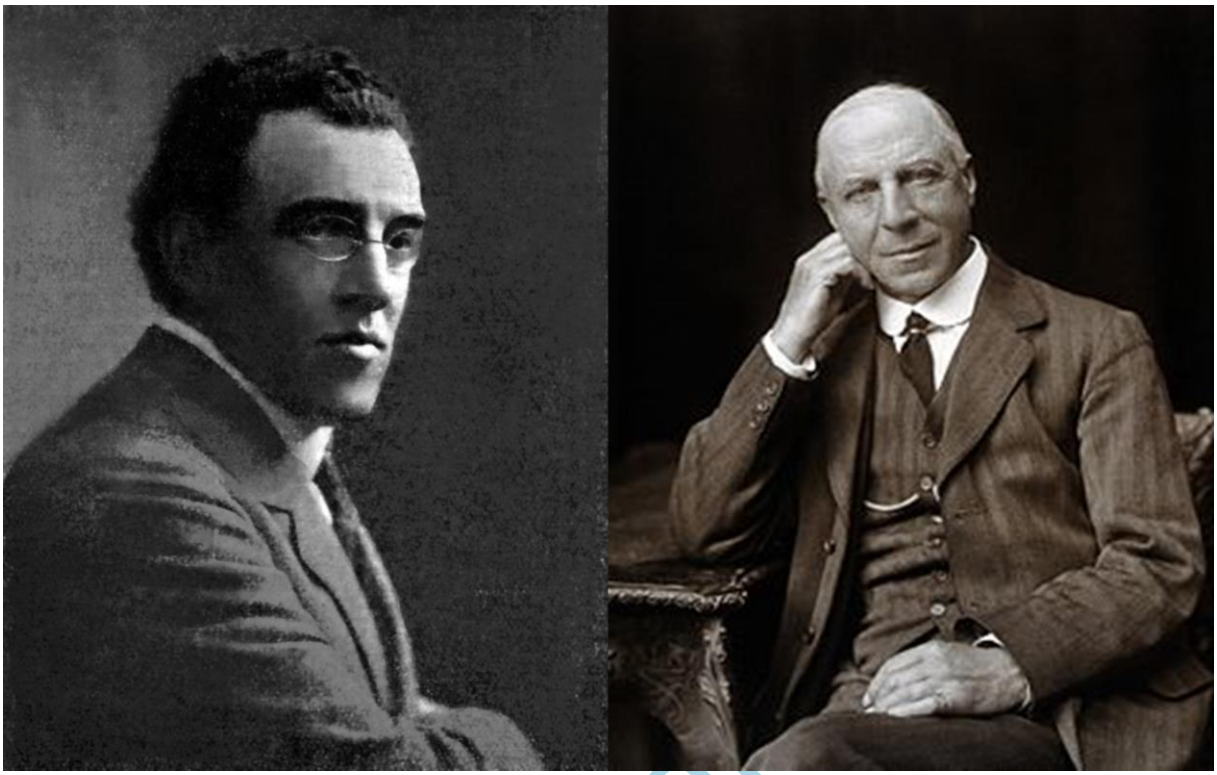


Figure 3

SURGERY, GYNECOLOGY AND OBSTETRICS

AN INTERNATIONAL MAGAZINE, PUBLISHED MONTHLY

VOLUME XXXI NOVEMBER, 1920 NUMBER 5

THE RELATION OF THE ISLETS OF LANGERHANS TO DIABETES WITH SPECIAL REFERENCE TO CASES OF PANCREATIC LITHIASIS

By MOSES BARRON, M.D., MINNEAPOLIS, MINNESOTA

From the Department of Pathology, University of Minnesota, Minneapolis, Minnesota

ANY reference to the pancreas as secreting a hormone necessary for the utilization of sugar by the tissues of the body is misleading, as that function is, accurately speaking, exercised by only a very small portion of the organ, the so-called "islets" of Langerhans; so that what is generally understood as the relation of the pancreas to diabetes is rather the relation of the islets to that disease. And yet it should not be overlooked that in spite of a great abundance of proof from experimental and clinical studies, it has not been universally accepted that the deficiency of either the pancreas as a whole or of the specific portion of it, the islets, results in diabetes mellitus.

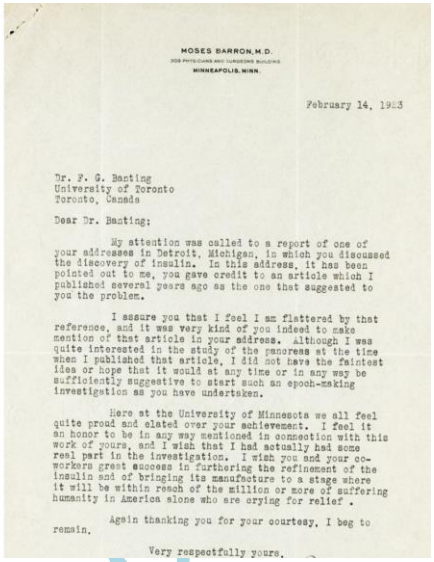
The purpose of this paper is to present examples of typical changes in the islets found in cases of true diabetes together with a detailed study of the histopathology found in a case of pancreatic lithiasis with special reference to the islets, and to correlate these findings with those recorded in the literature as obtained in experimental ligation of the ducts in animals. Such a combined study of clinical and experimental cases is of special advantage because of the similarity between the spontaneous and the induced conditions.

Pancreatic lithiasis is a very rare condition. Only a relatively small number of cases have been recorded in the literature, although Graaf speaks of it as early as 1667, and Morgagni and Cawley recognized the condition in 1765 and 1778 respectively. Opie (35) found two cases in 1,500 autopsies. Rindfleisch (39) found 3 cases in a series of 2,000 autopsies. Zesas (45) in 1903 collected only about 70 cases from the literature. Of these, 7 had been diagnosed clinically. Einhorn (10) states that the clinical recognition of this disease is exceedingly rare; much rarer than the very rare condition itself. In our own laboratory, this was the first case found in a series of several thousand autopsies.

Gall-stones are generally found in the gall-bladder; they are rare in the ducts. Pancreatic stones, on the other hand, are found lodging in the ducts in the absence of a cystic diverticulum. It is probable that pancreatic lithiasis is more common than is suspected, but the condition is not recognized unless the stones are large enough to meet resistance. Small stones may be expelled into the intestine without any symptoms. A few large stones have also been found in the feces (13), but this is very much rarer than in the case of gall-stones.

In contrast to the relative frequency of gall-stones in the female, more than 75 per cent of cases of pancreatic lithiasis occur in the male. Lazarus (23) collected 57 cases from the literature of which 47 occurred in the male. He states that about 60 per cent are found during the fourth decade.

437



MOSES BARRON, M.D.
308 PHYSICIANS AND SURGEONS BUILDING
MINNEAPOLIS, MINN.

February 14, 1923

Dr. F. G. Banting
University of Toronto
Toronto, Canada

Dear Dr. Banting:

My attention was called to a report of one of your addresses in Detroit, Michigan, in which you discussed the discovery of insulin. In this address, it has been pointed out to me, you gave credit to an article which I published several years ago as the one that suggested to you the problem.

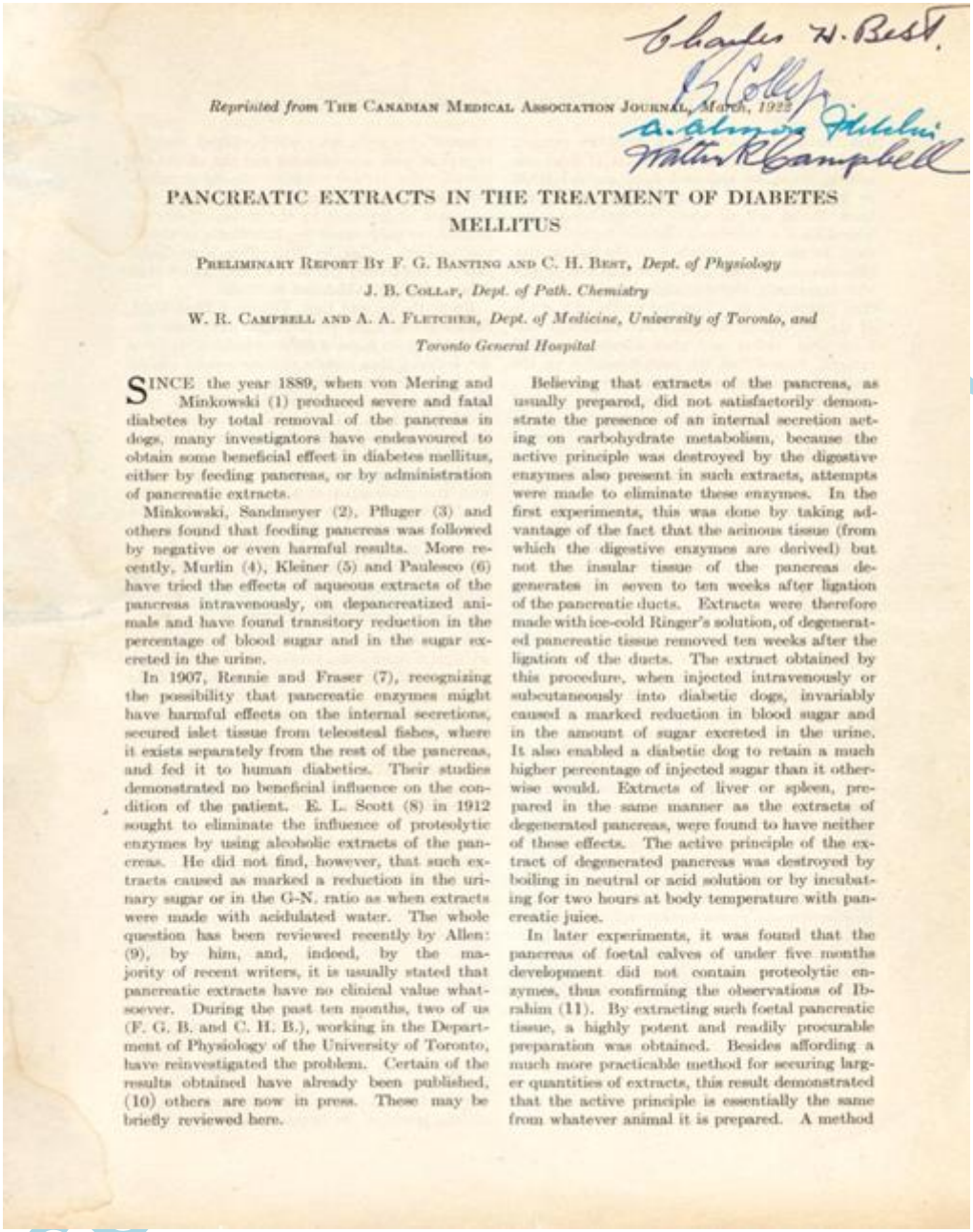
I assure you that I feel I am flattered by that reference, and it was very kind of you indeed to make mention of that article in your address. Although I was quite interested in the study of the pancreas at the time when I published that article, I did not have the faintest idea or hope that it would at any time or in any way be sufficiently suggestive to start such an epoch-making investigation as you have undertaken.

Here at the University of Minnesota we all feel quite proud and elated over your achievement. I feel it an honor to be in any way mentioned in connection with this work of yours, and I wish that I had actually had some real part in the investigation. I wish you and your co-workers great success in furthering the refinement of the insulin and of bringing its manufacture to a stage where it will be within reach of the million or more of suffering humanity in America alone who are crying for relief.

Again thanking you for your courtesy, I beg to remain,

Very respectfully yours,

Figure 4



Charles H. Best.
J. B. Collip
A. A. Fletcher
Walter R. Campbell

Reprinted from THE CANADIAN MEDICAL ASSOCIATION JOURNAL, March, 1922

PANCREATIC EXTRACTS IN THE TREATMENT OF DIABETES MELLITUS

PRELIMINARY REPORT BY F. G. BANTING AND C. H. BEST, *Dept. of Physiology*
J. B. COLLIP, *Dept. of Path. Chemistry*
W. R. CAMPBELL AND A. A. FLETCHER, *Dept. of Medicine, University of Toronto, and Toronto General Hospital*

SINCE the year 1889, when von Mering and Minkowski (1) produced severe and fatal diabetes by total removal of the pancreas in dogs, many investigators have endeavoured to obtain some beneficial effect in diabetes mellitus, either by feeding pancreas, or by administration of pancreatic extracts.

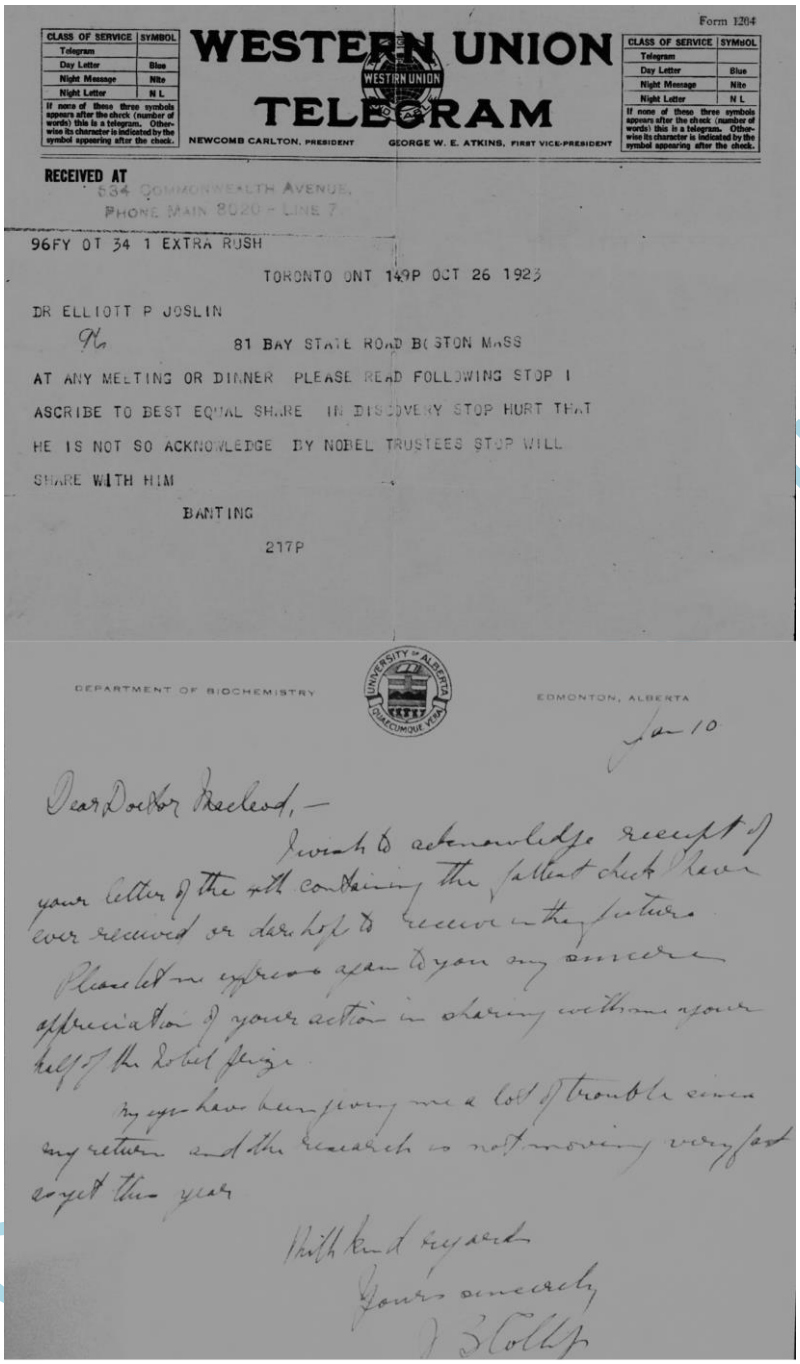
Minkowski, Sandmeyer (2), Pfluger (3) and others found that feeding pancreas was followed by negative or even harmful results. More recently, Murlin (4), Kleiner (5) and Paulesco (6) have tried the effects of aqueous extracts of the pancreas intravenously, on depancreatized animals and have found transitory reduction in the percentage of blood sugar and in the sugar excreted in the urine.

In 1907, Rennie and Fraser (7), recognizing the possibility that pancreatic enzymes might have harmful effects on the internal secretions, secured islet tissue from teleosteal fishes, where it exists separately from the rest of the pancreas, and fed it to human diabetics. Their studies demonstrated no beneficial influence on the condition of the patient. E. L. Scott (8) in 1912 sought to eliminate the influence of proteolytic enzymes by using alcoholic extracts of the pancreas. He did not find, however, that such extracts caused as marked a reduction in the urinary sugar or in the G-N. ratio as when extracts were made with acidulated water. The whole question has been reviewed recently by Allen: (9), by him, and, indeed, by the majority of recent writers, it is usually stated that pancreatic extracts have no clinical value whatsoever. During the past ten months, two of us (F. G. B. and C. H. B.), working in the Department of Physiology of the University of Toronto, have reinvestigated the problem. Certain of the results obtained have already been published, (10) others are now in press. These may be briefly reviewed here.

Believing that extracts of the pancreas, as usually prepared, did not satisfactorily demonstrate the presence of an internal secretion acting on carbohydrate metabolism, because the active principle was destroyed by the digestive enzymes also present in such extracts, attempts were made to eliminate these enzymes. In the first experiments, this was done by taking advantage of the fact that the acinous tissue (from which the digestive enzymes are derived) but not the insular tissue of the pancreas degenerates in seven to ten weeks after ligation of the pancreatic ducts. Extracts were therefore made with ice-cold Ringer's solution, of degenerated pancreatic tissue removed ten weeks after the ligation of the ducts. The extract obtained by this procedure, when injected intravenously or subcutaneously into diabetic dogs, invariably caused a marked reduction in blood sugar and in the amount of sugar excreted in the urine. It also enabled a diabetic dog to retain a much higher percentage of injected sugar than it otherwise would. Extracts of liver or spleen, prepared in the same manner as the extracts of degenerated pancreas, were found to have neither of these effects. The active principle of the extract of degenerated pancreas was destroyed by boiling in neutral or acid solution or by incubating for two hours at body temperature with pancreatic juice.

In later experiments, it was found that the pancreas of foetal calves of under five months development did not contain proteolytic enzymes, thus confirming the observations of Ibrahim (11). By extracting such foetal pancreatic tissue, a highly potent and readily procurable preparation was obtained. Besides affording a much more practicable method for securing larger quantities of extracts, this result demonstrated that the active principle is essentially the same from whatever animal it is prepared. A method

Figure 5



Form 1204

CLASS OF SERVICE	SYMBOL
Telegram	
Day Letter	Blue
Night Message	Nite
Night Letter	N L

If none of these three symbols appears after the check (number of words) this is a telegram. Otherwise its character is indicated by the symbol appearing after the check.

WESTERN UNION

TELEGRAM

NEWCOMB CARLTON, PRESIDENT GEORGE W. E. ATKINS, FIRST VICE-PRESIDENT

RECEIVED AT
534 COMMONWEALTH AVENUE,
PHONE MAIN 8020 - LINE 7.

96FY OT 34 1 EXTRA RUSH

TORONTO ONT 149P OCT 26 1923

DR ELLIOTT P JOSLIN

81 BAY STATE ROAD BOSTON MASS

AT ANY MEETING OR DINNER PLEASE READ FOLLOWING STOP I ASCRIBE TO BEST EQUAL SHARE IN DISCOVERY STOP HURT THAT HE IS NOT SO ACKNOWLEDGE BY NOBEL TRUSTEES STOP WILL SHARE WITH HIM

BANTING

217P

DEPARTMENT OF BIOCHEMISTRY

EDMONTON, ALBERTA

Jan 10

Dear Doctor Macleod, —

I wish to acknowledge receipt of your letter of the 9th containing the fullest check I have ever received or dare hope to receive in the future. Please let me express again to you my sincere appreciation of your action in sharing with me your half of the Nobel prize.

I fear I have been giving you a lot of trouble since my return and the research is not moving very fast so yet this year.

With kind regards

Yours sincerely

J Hollis

Figure 6

Figure 7

Figure 8