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Banting and Best: The Extraordinary Discovery of Insulin

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A B S T R A C T

Diabetes was a feared disease that most certainly led to death before insulin discovery. During the first two decades of the 20th century, several researchers tested pancreatic extracts, but most of them caused toxic reactions impeding human use. On May 1921, Banting, a young surgeon, and Best, a master's student, started testing the hypothesis that, by ligating the pancreatic ducts to induce atrophy of the exocrine pancreas and minimizing the effect of digestive enzymes, it would be possible to isolate the internal secretion of the pancreas. The research took place at the Department of Physiology of the University of Toronto under supervision of the notorious physiologist John MacLeod. Banting and Best felt several difficulties depancreatising dogs and a couple of weeks after the experiments had begun most of the dogs initially allocated to the project had succumbed to perioperative complications. When they had depancreatised dogs available, they moved to the next phase of the project and prepared pancreatic extracts from ligated atrophied pancreas. These extracts effectively reduced glycaemia on dogs. However, duct ligation was so time and animal consuming, that Banting and Best looked for alternatives and developed a method of extraction without the ligation procedure from fresh pancreata, which were readily available. These extracts were shown to be equally effective. Meanwhile, Collip, a biochemist of the University of Alberta, joined the group and developed a method for purifying insulin, which allowed its widespread human use. On 11 January 1922, Leonard Thomson, a 14–year–old boy with type 1 diabetes, received the first subcutaneous injections of Banting and Best's extracts. On October 1923, the Nobel Prize in Physiology or Medicine was awarded to Banting and Macleod for the discovery of insulin. In disagreement with the Karolinska Nobel Committee, Banting shared his prize with Best and MacLeod shared his prize with Collip.

Banting e Best: A Extraordinária Descoberta da Insulina

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R E S U M O

Antes da descoberta da insulina a diabetes era uma doença temida, que impreterivelmente conduzia à morte. Durante as duas primeiras décadas do século XX, vários investigadores testaram extratos pancreáticos, porém a maioria deles provocava reações tóxicas graves que impediam a sua utilização. Em maio de 1921, Banting, um jovem cirurgião, e Best, um estudante de Medicina, começaram a testar a hipótese de que a ligação dos ductos pancreáticos induziria a atrofia do pâncreas exócrino, minimizando o efeito das enzimas digestivas, e permitindo isolar a secreção interna do pâncreas. A investigação realizou-se no Departamento de Fisiologia da Universidade de Toronto, sob orientação do reconhecido fisiologista John MacLeod. Banting e Best tiveram dificuldades em realizar pancreatectomias e, após duas semanas de experiências, a maioria dos cães que MacLeod atribuíra ao projeto tinham morrido de complicações perioperatórias. Depois de alcançarem sucesso nas pancreatectomias, Banting e Best iniciaram a preparação de extratos a partir de pâncreas com ductos previamente ligados. Estes extratos reduziam eficazmente a glicemia em cães. No entanto, a ligação dos ductos exigia bastante tempo e animais, pelo que procuraram outras alternativas. Para tal, desenvolveram um método de extração a partir pâncreas frescos, que estavam facilmente disponíveis e não necessitavam da ligação dos ductos. Estes extratos mostraram-se igualmente eficazes. Entretanto, Collip, um distinguido bioquímico da Universidade de Alberta, juntou-se ao grupo e desenvolveu um método para purificar a insulina, que

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permitiu o seu uso generalizado. Em 11 de janeiro de 1922, Leonard Thomson, um jovem de 14 anos com diabetes tipo 1, recebeu as primeiras injeções subcutâneas de extratos de insulina. Em outubro de 1923, Banting e Macleod foram galardoados com o Prémio Nobel de Fisiologia ou Medicina pela descoberta da insulina. Em desacordo com o Comité Nobel do Instituto Karolinska, Banting dividiu seu prémio com Best e MacLeod com Collip.

Introduction

During the 18th and 19th centuries, the role of pancreas in glucose homeostasis went through remarkable advances. In 1815, the French chemist Michel Chevreul (1786–1889) showed that the sugar present in the urine of patients with diabetes was glucose.[1] In 1893, Gustave Édouard Laguesse (1861–1927) suggested that the cell clusters dispersed in the pancreatic parenchyma would form the endocrine tissue and have them called "islets of Langerhans", honouring Paul Langerhans that described them 25 years earlier.[2] In 1901, Eugene Lindsay Opie (1873–1971) published his studies on pancreatic microscopy of patients with diabetes.[3,4] He observed hyaline degeneration in the islets of Langerhans of a 17–year–old girl with diabetes for 2 years.[4] Later, in 1910, he compiled more than 400 works in his book: "Disease of the Pancreas: Its Cause and Nature".[5] Leonid Ssobolew (1876–1919), who had inspired Opie's work, reported partial and, in some cases, total absence of islets of Langerhans in 13 cases of fatal diabetes.[6]

Therefore, the evidence implicating the islets of Langerhans in the pathogenesis of diabetes was emerging and the following years were of intense research. In 1909, Jean De Meyer (1878–1934), a Belgian physician, postulated, long before the antidiabetic hormone was discovered, that the hypoglycaemic hormone was produced by the islets of Langerhans and have it called *insulin* (derived from *insula* Latin).[7] A few years later, in 1916, Edward Albert Sharpey–Schäfer (1850–1935), a British physiologist and physician, independently coined the same name for the antidiabetic hormone.[8]

The discovery of insulin did not emerge from vacuum. Between the late 19th and early 20th centuries, many researchers had devoted them to study the antidiabetic agent. It was estimated that between 1890–1910, about 400 researchers tried to treat diabetes with pancreatic extracts.[9] The research of Frederick Banting, Charles Best, John MacLeod, and James Collip culminating with the successful administration of pancreatic extracts to humans will be reviewed here (Fig. 1).

Frederick Grant Banting (1891–1941)

Frederick Grant Banting was born on 14 November 1891. He was the fifth son of William and Margaret Banting, a family of Irish and Scottish descent, respectively. Banting studied at the local public school, in Allinston, and he was more successful on sports than on school.[10] In the fall of 1912, Banting enrolled the Medical School at the University of Toronto. Despite his class (1T7) was scheduled to graduate in 1917, his course was shortened because of the war and the fifth year of the medical course was taught during the summer of 1916.[11] In 1917, he was sent to the front line with the Canadian Army Medical Corps and, on 28 September 1918, he was wounded at the Battle of Cambrai, in the northern of France.[10] After the war he continued his training as a Surgery registrar under Clarence Starr supervision at the Hospital for Sick Children, in Toronto (1919–1920), where he developed a special

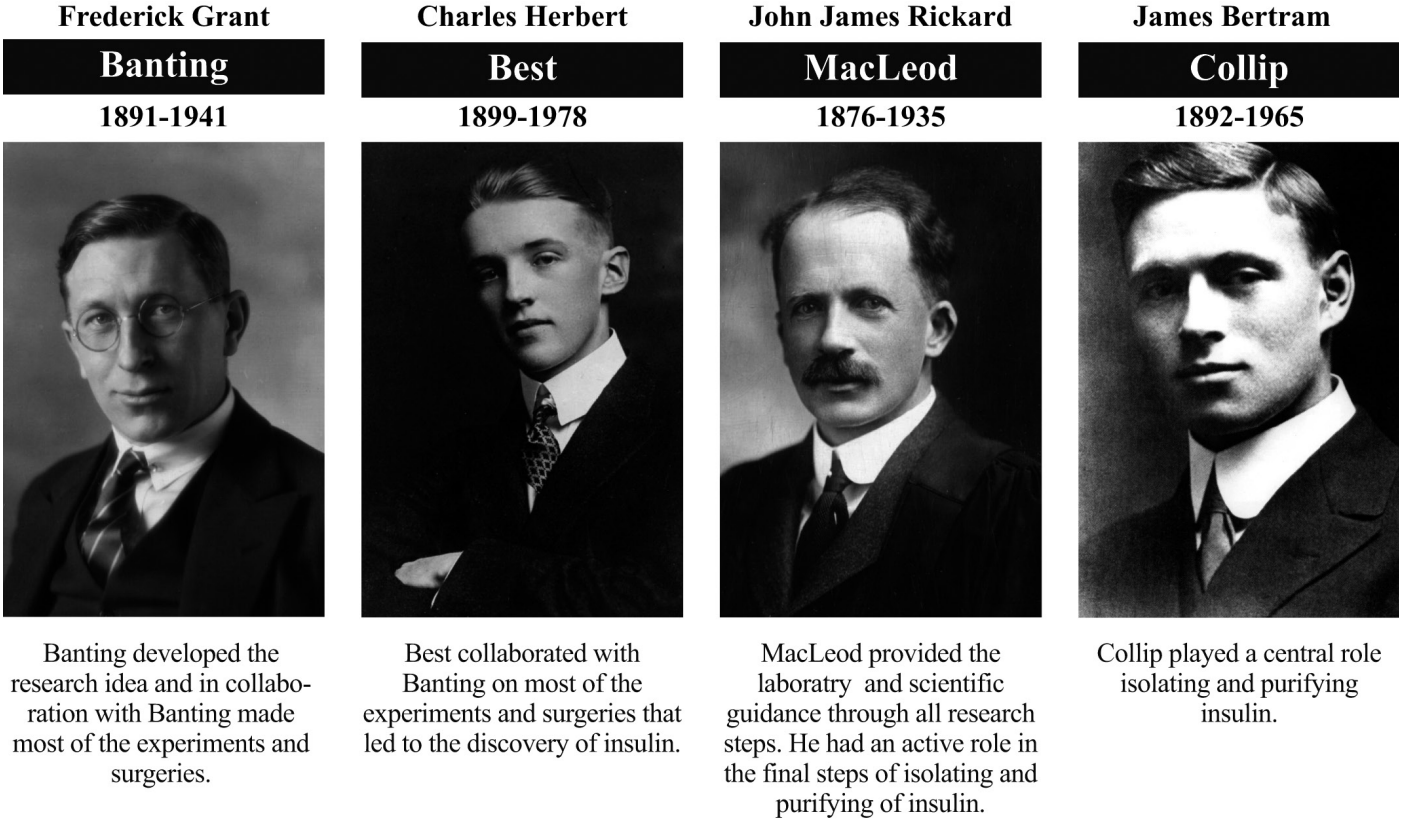

Figure 1. The Toronto group involved in the discovery of insulin.

interest on Orthopaedics. However, he was not able to secure a position at the hospital in 1920 and he moved elsewhere.[12]

On 1 July 1920, at the age of 29, Banting settled in London, a small town in Ontario, Canada. He started there his private practice, however, without much success. The first patient he attended came on 29 July, a former military, looking for an alcohol prescription because he would have friends coming over that weekend and he wanted to give them a drink.[A,12] That month he has earned only $4. To idle away the time, he built a garage and repaired a Ford he bought in fourth or fifth hand. Banting's life was not easy and he was going through great financial difficulties.

In October 1920, he applied for a part–time job as a demonstrator of anatomy and surgery at the Faculty of Medicine of University of Western Ontario. He was paid about $2/hour.[13] He met the physiologist Frederick Miller (1881–1967) and soon he offered his help, as part–time assistant, on the research of cerebellum physiology.[14] Additionally, he was involved in teaching physiology to medical students. The idea of pancreatic duct ligation, causing exocrine pancreas atrophy, and therefore facilitating the extraction of the internal secretion came to him while preparing a talk for physiology students on carbohydrate metabolism. This idea eventually led to a chain of events that changed his life forever and ended with the discovery of insulin, in 1922.

In mid–March 1921, Banting's relationship with his girlfriend, Eddy Roach, had irreversible degraded. He was again in extreme financial difficulties, therefore when he heard that an expedition going to Mackenzie River to search for oil was looking for a medical officer to accompany them, he decided to leave his fate on the toss of a coin: "Heads I was to do the research, tails I was to go to the Arctic to search for oil".[12] Artic won, in three out of five tosses came up tails. However, a few weeks later he was informed that the expedition would not take a physician on board after all.[15] After failing to arrange a job, Banting made intensive efforts to secure the start his research project on pancreatic diabetes in May 1922.

The events and experiments leading to the insulin discovery are discussed in the next sections. In 1923, after the discovery of insulin, Banting was appointed Chair of the Banting Institute, a position he retained until his death. During the same period, he was appointed honorary consulting physician at the Toronto General Hospital, the Hospital for Sick Children, and the Toronto Western Hospital.[10] He researched other areas, such as cancer, silicosis, drowning, and flight disease.[11,16-18]

In 1924, Banting got married with Marion Robertson, a radiology technician at Toronto General Hospital, with whom he had a son in 1928. His marriage was very problematic and he got divorced in 1932. Later, in 1937, he married again to Henrietta Ball, a technician from his department.[10,11]

In the last years of his life, Banting had developed a growing animosity toward Best. He felt particularly exasperated when Best who, during World War II, had been lobbying to be appointed the Canadian medical expert for a liaison mission between Britain and North America, refused to make a trip to the United Kingdom.[19] Aware of the risks, Banting decided that himself would take Best's place. The flight occurred on 21 February 1941, but unfortunately the plane crashed in Newfoundland, Canada, resulting in Banting's death.

Charles Herbert Best (1899–1978)

Charles Herbert Best, son of Herbert Huestis Best and Luella Fisher, was born on 27 February 1899, in West Pembroke, Maine, United States. He spent part of his childhood and education there, and after his high school graduation, in 1915, he moved to Toronto, Canada.[20] Best temporarily attended Harbord Collegiate to prepare his University application and, in 1916, he enrolled the Bachelors of Arts on biochemistry and physiology at the University of Toronto.[21] Regrettably, war time also affected Best and, in 1918, he interrupted his studies to join the Canadian army. He served in Petawawa, Ontario, on the 70th Battery of the Horse Artillery, as sergeant. Later he went to Wales, as part of a draft for the 2nd Canadian Tank Battalion.[20]

In 1919, after military service, Best returned to Toronto and resumed his studies. In June 1921, with 22 years old, he was awarded a Bachelors of Arts degree in physiology and biochemistry.[22]

Best and his classmate, and close friend, Clark Noble (1900–1978) started that summer a Master of Arts degree at the University of Toronto in the laboratory of MacLeod.[23] Since his youth, Best was very interested in diabetes and metabolism of carbohydrate metabolism research. When he was 15 years old, Best was deeply touched by his aunt Anna Best death. She had been treated by Elliott Joslin for diabetes with the undernutrition regimen. After moving to Pembroke, to stay with the Bests, Anna died in diabetic coma, in 1918.[24] Noble and Best were considered by MacLeod to assist Banting in his research. According to reports, they had decided who would start the first 4–week period by a coin toss. Best won the toss, and after four weeks he would be replaced by Noble. However, Best proved to be proficient in assisting Banting in the surgical technique, therefore they agreed that was in the best interest of the research that Best remained full time with Banting.[23]

In 1925, Best was awarded with his Medical Doctor degree. Between 1925 and 1929, he decided to move to Europe, where he continued his research at the University of Freiburg and at the University of London.

Best and Noble's friendship survived the discovery of insulin, but it did not survive the turmoil that settled thereafter. Noble was an usher at Best and Margaret Hooper Mahon's wedding that took place on 3 September 1924. He had met his wife four years before at Noble's family farm. Unfortunately, the contacts between Best and Noble became sporadic, and on 16 June 1926, Best did not attend Noble's wedding because he was in London at that time.[23]

In addition to insulin, Best dedicated himself to research blood transfusion during World War II, he discovered choline, and introduced heparin as treatment for thrombosis.[21] Best died, in Toronto, on 3 March 1978 at the age of 79.

The Discovery of Insulin

The idea

At 02.00 hours of 31 October 1920, while preparing a talk he had to give to physiology students about a subject he was not familiar with, Banting read Barron Moses article: "The relation of the Islets of Langerhans to diabetes with special reference to cases of Pancreatic lithiasis" and wrote in his book notes (Fig. 2):[25]
"Diabetus (sic)

Ligate pancreatic ducts of the dog. Keep dogs alive till acini degenerate leaving Islets. Try to isolate the internal secretion of these to relieve glycosurea".[26]

The next day he reported his idea to Miller, and because the Western's Department of Physiology was not equipped for that kind of research, he advised Banting to discuss the matter with

[A] By that time, in Canada, alcohol was only sold for medical purposes.

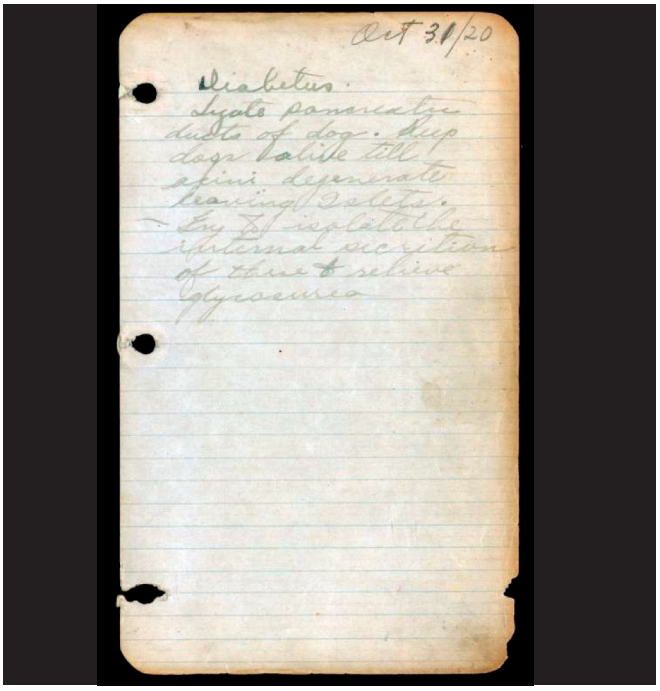

Figure 2. Banting's notebook record on the duct–ligation method for research of pancreatic internal secretions.

the specialist on metabolism of carbohydrates John James Rickard Macleod (1876–1935) at the University of Toronto.[9] The meeting took place on 7 November 1920.[10] MacLeod soon realized that the young Banting knew little about the pancreas, diabetes, or even the basic research methodology he intended to accomplish.[12] Nevertheless, after Banting persistence, Macleod agreed to provide a small unused room at the Department of Physiology and the assistance of a medical student. The experiments would begin at the end of the medical term, on May 1921, with the help of an assistant and 10 dogs.[27]

Banting was not the first to propose that it would be possible to obtain the internal secretion of the pancreas through the duct ligation method. In 1902, Ssobolew had already proposed it.[6] However, as the studies of Milne and Peters demonstrated, some islet degeneration occurred, as well as some exocrine acini preservation occurred.[28] In contrast to Banting and MacLeod's believe, and as shown by the German physiologist Rudolf Heidenhain in 1875, pancreatic extracts do not contain active proteolytic enzymes that would digest the internal secretions.[29,30] The "zymogen which under certain conditions forms an active ferment" is activated when a pancreas is cut out and begins to deteriorate, however, this happens slowly and can be prevented by chilling.[29,31] Roberts, a physiologist at Cambridge University, criticize deeply this misconception because "it is one of the best established facts in physiology that the proteolytic enzyme exists in the pancreas in an inactive form – trypsinogen – which is activated normally only on contact with another ferment, enterokinase, secreted by the small intestine".[32] And he concludes that "the production of insulin originated in a wrongly conceived, wrongly conducted, and wrongly interpreted series of experiments… I venture to believe that whatever success the remedy will have will be found to be due to the fact that the hormone has been obtained free from anaphylaxis–producing and other toxic substances". Henry Hallett Dale (1875–1968) reacted to the harsh tone of Roberts' words and added that "it is a poor thing to attempt belittlement of a great achievement by scornful exposure of errors in its inception".[33]

The development of experimental diabetes

On 14 May 1921, Banting visited the room where he would be working in the next weeks. The room looked more like a warehouse than a laboratory. Best had his last examination on 16 May and, in the following morning, he and Banting cleaned and prepared themselves the room to become better suited for research.[34] On 17 May, in the presence of MacLeod, Banting performed the first pancreatectomy.[10] At that time, pancreatectomy was mainly a research procedure and possibly Banting had never done it before.

MacLeod decided to perform Hédon's two–step method: the first step included removing most of the pancreas, but keeping a small pedicle that was fixed in the abdominal subcutaneous tissue; the second step, should be done a week later, when the animal was recovered, and included the extraction of the pedicle, therefore completing the pancreatectomy and making the animal diabetic.[35] The first surgery was performed to a cocker spaniel (dog n.º 385). It took approximately 80 minutes to complete the first step. On 20 May, three days after the procedure, the dog died possibly due to wound infection.[11]

On 18 May, Banting and Best started their epic self–learning journey depancreatising dogs. That morning they did their first surgery alone. The dog died from anaesthetic overdose. The next dog was very small and Banting did not properly clamp the blood vessels, which led to the dog's death from bleeding immediately after surgery. On 19 May, the dog nº 386, survived the first step of Hédon's procedure, but died on Saturday, 21 May 1921.[11] The four animals operated during the first week died from surgical complications. That same Saturday, Banting operated more carefully a fifth dog (nº 387) and fixed the pancreatic pedicle closer to the skin in order to facilitate drainage.[11,36]

The dog nº 387 was recovering well. Therefore, Banting and Best felt that was time to move to the next phase of the experiment: pancreatic duct ligation, exocrine pancreas atrophy, and production of internal secretion extracts. The surgical technique was again challenging to the team, and the first dog undergoing duct ligation died three days after the surgery from infection, the second survived the surgery but Banting failed to ligate the pancreatic ducts, and the third dog died two days after surgery also from infection.[11] In the first two weeks of experiments seven out of ten operated dogs were death and Banting was worried about the mortality rate. In the following week, he gave a dog the same number of other one that had just died. In fact, dog's double numbering is seen in other occasions in the Banting's notebook during the summer of 1921.[11] By this time, they had already exhausted the 10 dogs provided by MacLeod and had no funding from the University, therefore they decided to use their own funding and began buying dogs on the streets for $1–3.[11,37]

Dog nº 387 was recovering well from the first–step of Hédon's technique and was tested normal for glycosuria and blood glucose levels using the Myers–Bailey modification of the Lewis–Benedict method.[B] On 28 May, Banting removed the remaining pedicle and completed the pancreatectomy for the first time.[36] Diabetes soon manifested, and glycosuria and blood glucose concentration of 0.35% were observed. The dog died on 1 June from infection found at the autopsy.

In June, MacLeod travelled to Europe on holidays but not before leaving a series of methodological instructions. According

[B] This technique had proven to be advantageous and required much less blood then the procedures that John Rennie (1865–1928), Nicolae Constantin Paulescu (1869–1931), and others before them had used.[9,38,39]

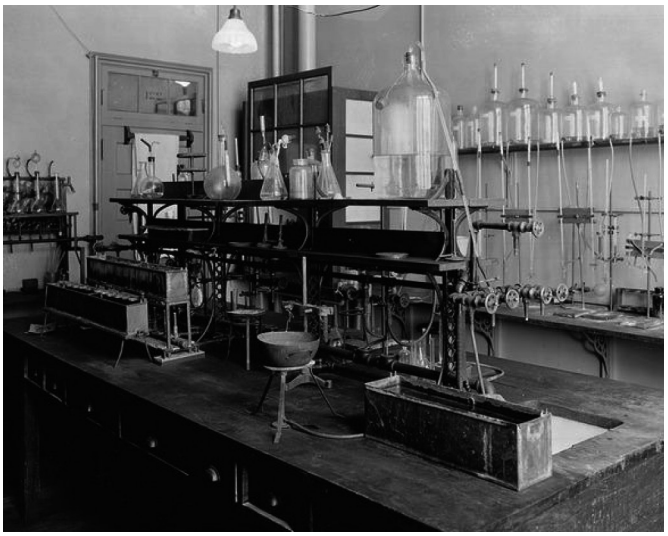

Figure 3. Laboratory 221 where Banting and Best undertook most of their research.

to Banting's notes: "have dogs diabetic DN ratio constant for 3 days. meat diet, (l) intra peritoneal graft (2) subcutaneous graft (3) whole intravenous injection (4) Divided aq. 2 hours intravenous (5) subcutaneous injection". Additionally, he suggested to keep "microscopic sections of remnant before and after transplant", and that glucose (50 g) should be given "with whole gland remnant".[11]

On the first half of June, Banting and Best had two dogs with the first step of Hédon's procedure completed and seven dogs with the pancreatic ducts ligated. On 15 of June, Banting successfully completed a pancreatectomy for the second time. He recorded this success on the dog nº 386![C] In the following weeks Banting and Best experienced multiple failures on dog's pancreatectomy and ligation procedures.

The fact that surgeries were performed in a small room, previously used as a storage, right next to the animal facility and laboratory did not guarantee the best surgical environment (Fig. 3). In July, Toronto's high temperature and humidity were not the best conditions for healing and recovery of the operated animals, and only two out of the seven ligated dogs showed pancreatic degeneration, while both dogs that underwent pancreatectomy died from infection.

[C] Number previously used to identify a different dog.

Experiments with pancreatic extracts

Banting and Best's pancreatectomy success rate improved and, on 30 July 1921, they decided to treat depancreatised dogs with extracts prepared from pancreas of dogs with ligated ducts.[36] The degenerated pancreas from the dog nº 391, previously ligated on 7 June, was removed and prepared for intravenous administration. At 10:15 hours, on 30 July, the dog nº 410 received the first intravenous administration of pancreatic extracts. Glucose concentration decreased from 0.2% to 0.12% in the first hour. However, another injection (5 cc) at 11.00 am caused only a mild glucose decrease to 0.11%, and another at around 12:00 hours had no evident effect on glucose levels and at 14:15 it was starting to rise to 0.14%. Furthermore, the hypoglycaemic effect was not sustained, particularly when glucose boluses were given by stomach tube (Fig. 4).[11] In the next day, the dog was found in a coma, and the last glucose measurement before dog's death was 0.15%. Unfortunately, no autopsy was done and no cause of death was determined.

On 1 August, the last depancreatised dog (nº 406) available was in a coma, with a blood glucose concentration of 0.5%, and therefore 8 cc of pancreatic extracts were given intravenously. One hour later glucose had decreased to 0.42% and, amazingly, the dog came out of coma and was able to walk. Another 5 cc of extract was given and one hour later blood glucose was 0.3%. Unfortunately, the dog died approximately three hours after receiving the first extract (last blood glucose measurement was 0.37%). Again, the cause of death was unknown because no autopsy was performed.

Taking in consideration the absence of depancreatised dogs, the difficulties in acquiring new dogs for experiments, and the time required to perform the two–step of Hédon's pancreatectomy,

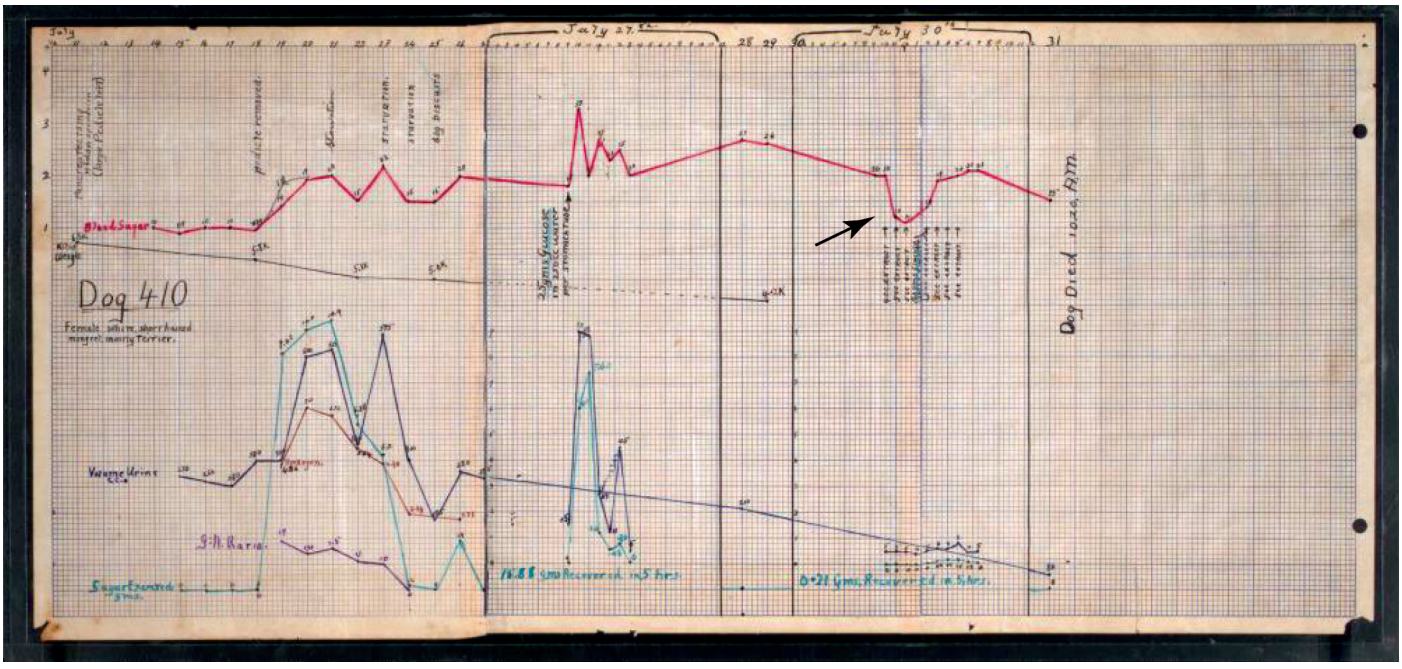

Figure 4. Variation of glycaemia (arrow) of the first dog (nº 410) treated with intravenous insulin extracts, on 30 July 1921.

Best suggested to attempt total pancreatectomy in a single surgical time. On 3 August, the dog nº 408 underwent total pancreatectomy at a single surgery procedure and, for Banting's amazement, the procedure was even easier to perform than the Hédon's method and the dog survived. In the following afternoon, 5 cc of pancreatic extracts were injected by intravenous route and in 35 minutes blood glucose decreased from 0.26% to 0.16%. On 4 August 1921, Banting recorded, for the first time, in his notebook, the term *isletin* referring to the pancreatic extract given to the dog nº 408 (Fig. 5).[40] They also tried other extracts (liver and spleen) without success and, on 7 August, the dog died from peritonitis secondary to the surgery.

On 9 August, Banting and Best wrote to MacLeod informing about their progress and, on 11 August, they decided to perform total pancreatectomy at a single surgical time on dogs nº 92 and nº 409. The former would be treated with *isletin*, while the latter would not receive any treatment at all. Four days after the pancreatectomy the dog nº 409 died, while the dog n.º 92 survived until 31 August. The shortage of pancreatic extracts produced from atrophied pancreas led Banting and Best to prepare extracts from fresh pancreas that had not been ligated. These extracts were given to dog nº 92. Blood glucose decreased from 0.30% to 0.17% one hour after administration of 10 cc of these extracts. However, the authors wrongly concluded that: "It is obvious from the chart that the whole gland extract is much weaker than that from the degenerated gland" (Fig. 6).[40] They also tried another extraction method using continuous secretin stimulation, leading to exocrine

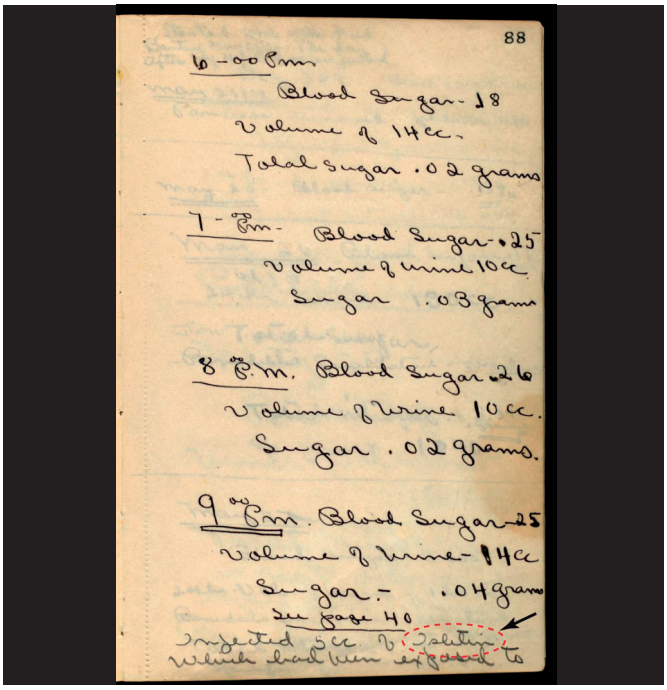

Figure 5. The word *isletin* (dashed circle) was used by Banting for the first time, on 4 August 1921, to designate the pancreatic extracts administered to the dog nº 408. Later, at MacLeod's suggestion, the name was changed to insulin, from the Latin word *insula*.

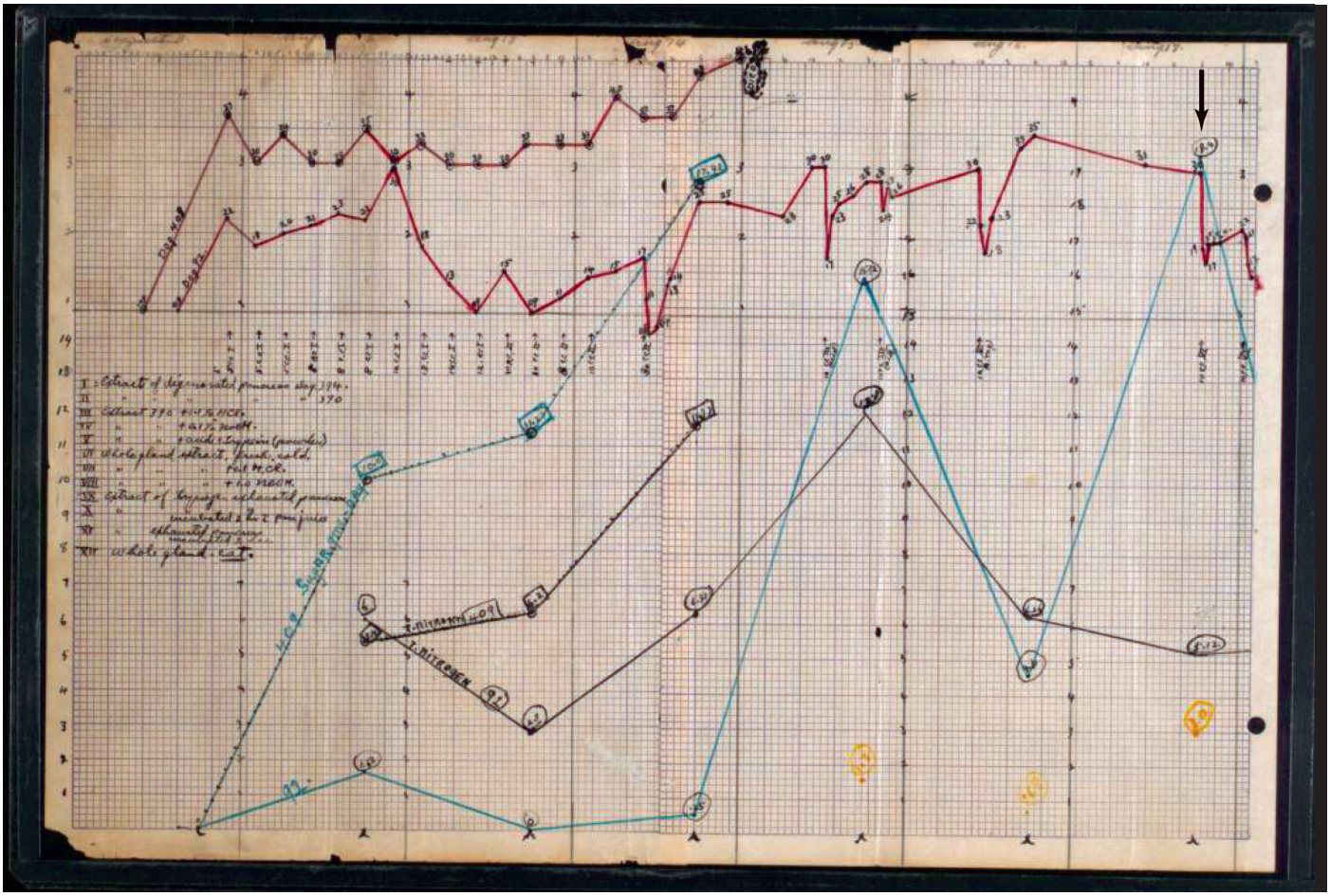

Figure 6. Variation of glycaemia of dog nº 406 (not treated with *isletin*) and dog nº 92 (treated with *isletin*). Banting and Best did not recognize that the administration of extracts of fresh pancreas produced similar effects on glycaemia (arrow) as those from atrophied pancreas. This resulted in some delay on the research, but on late November or early December 1921, they realised that both methods of preparing extracts were equally effective.

pancreatic exhaustion and subsequent extract preparation from the whole pancreas. On 20 August 1921, this extract was given to dog nº 92 with great improvement. In September 1921, Banting and Best carried on more experiments with secretin–stimulation method, but they eventually realised that the results were not satisfactory. Additionally, they tried other routes of administration including rectal and, for the first time, the subcutaneous route. However, they noted toxic reactions on the site of injection and as Banting recorded in his notebook "no more subcutaneous injection till we get a trypsin free extract".[11]

On 21 September, Macleod returned from Europe and expressed some concerns on the results. He wanted Banting and Best excluded the effect of the extract volume haemodilution on the observed glucose fluctuations. Furthermore, they did not give intravenous glucose to the dogs treated with pancreatic extracts as he previously had suggested they should do.

In late September or early October, Banting had a fierce argument with MacLeod, where he made four demands: a salary (Banting was working for free since the beginning of the project and going through financial difficulties); a proper room to work; an employee to deal with the dogs; and repairs on the operating room floor. MacLeod was very apprehensive because the team had already largely exceeded the number of dogs previously agreed up on. Nevertheless, MacLeod bowed to pressure of Banting's threats of taking the research to another university, and ultimately agreed to their requests.

During the following weeks, Banting and Best undertook several experiments to answer the questions raised by MacLeod. By this time, they still had a high surgical mortality rate but they felt it was time to move forward and attempted to move their focus to keep dogs alive for long periods under extracts treatment – it was the *longevity experience*. The best known dog of this experience, Marjorie (nº 33), was killed after being treated with subcutaneous pancreatic extracts ~70 days (from 18 November to 27 January). The reason pointed out for Marjorie's sacrifice was the shortage of extracts, which needed to be directed to other projects. Marjorie suffered several abscesses at the insulin injection sites.[D]

Even after dog 92 experiment, Banting and Best still thought it was necessary to get rid of external pancreas to produce an effective antidiabetic ingredient. Nevertheless, they were looking for alternative sources of pancreatic extracts that did not require the lengthy procedure of duct ligation.[E] In November, they produced pancreatic extracts from foetal calf pancreas and obviated the duct–ligation procedure. In December, they successfully extracted the active ingredient with alcohol from dog's fresh pancreas, therefore obviating the requirement of the less accessible foetal pancreases.[11] These data finally convinced Banting and Best that duct–ligation procedure wasn't necessary.

On 14 November, Banting and Best presented their results to a small group of doctors from Toronto, at the Journal Club of the Department of Physiology of the University of Toronto.[36,41] By this time, Banting suggested that more people should be involved in the project, and in mid–December 1921, James Bertram Collip (1892–1965), a biochemist of the University of Alberta, who was serving part of his sabbatical at the Department of Pathology Chemistry Toronto, joined the group.[42] Collip was familiar with Paulescu's work and made important contributions to the insulin purification process.[43]

The first human trials

Joseph Gilchrist, Banting's friend and classmate, was the first patient with diabetes to receive insulin extracts. On 20 December 1921, they gave him a potent extract by oral route, but on the next day they concluded that there was "no beneficial result".[44]

MacLeod, that in 1921 was President of the American Association of Physiology, asked Banting to report their preliminary results at the Association's 34th annual meeting, held on 30 December 1921 at the Yale University, New Haven. The presentation's title was "The beneficial influences of Certain pancreatic extracts on pancreatic diabetes", and its summary was published in the American Journal of Physiology.[30,42] Banting had difficulty with public speaking and as he later recognised "I did not present it well". Therefore, during the discussion MacLeod assumed a leading role in rebutting the critics. Most questions were directed to the toxic effects of the extracts and to what extent their work was different from Zuelzer, Scott, Klein, and Paulescu. However, Banting and Best's records on adverse reactions were very poor (e.g. temperature was recorded in a single dog: 40°C). Therefore, MacLeod focused his responses on the effect of the extracts in decreasing glycaemia and "on the prolongation of life of two treated animals", one of which later survived 70 days (Marjorie's experiment was going on for five weeks at the time of the meeting).[11] George Clowes from the pharmaceutical company Eli Lilly, that was attending the meeting, offered his assistance for the experiments.

In January of 1922, excited by the good results of the extracts prepared by Collip and administrated to dogs and rabbits, Banting proposed MacLeod to start human trials. The first human injection of Banting and Best's extracts was given to Leonard Thomson, in the afternoon of 11 January 1922, by the House officer at the Toronto General Hospital, Ed Jeffrey (Fig. 7).[11,13] The boy was 14–year–old, weighting 27 kg, with positive glycosuria, ketonuria, and acetone breath. Banting had convinced MacLeod to authorize the extract preparation according to the methodology they developed. However, they included some of the Collip's purification concepts he was working on. Collip was not consulted in advance and felt that Banting and Best had unfairly appropriated his ideas. Leonard Thomson, that was in a diabetic coma, received two injections of 7.5 cc of pancreatic extract into each buttock. The effect of the first administration was modest: blood glucose decreased from 0.44% to 0.32%, 24–hour glycosuria decreased from 84 g in 4060 cc of urine to 91.5 g in 3625 cc of urine and ketonuria remained positive, but total acetone bodies decreased from 188 to 69 mg/L.[45] The absence of clinical improvement and the development of local inflammatory effects, with sterile abscess formation at the injection sites, led to the treatment suspension.[11,36]

The pressure on Collip was increasing and after days of intense work, he prepared a purer extract allowing the treatment of the young Leonard to be resumed. Daily injections were given from 23 January to 4 February 1922 with much better results: blood glucose decreased from 0.52% to 0.12% in 24 hours and ketonuria resolved completely.[45] Leonard Thompson received insulin for over 13 years until he died of pneumonia at the age of 27, and Ted Ryder, other of the first patients receiving insulin in Toronto, died in 1993 after 73 years.[27]

[D] Unfortunately, Banting and Best records on Marjorie experiment were very poor, and a small amount (3 mm) of pancreatic tissue was found in the duodenum and substantially decreased the importance of Marjorie.[11]

[E] Banting and Best had previously tried the secretin–stimulation method without much success, therefore they decided to return to the idea of using whole fresh pancreas, without duct ligation or secretin stimulation, that had previously shown success on dog n.º 92.

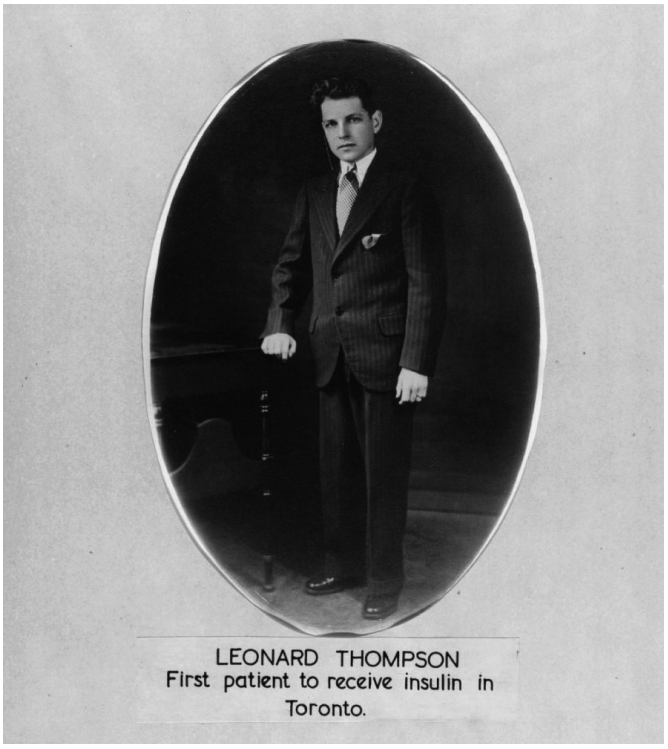

Figure 7. On 11 January 1922, Leonard Thomson was the first patient receiving Banting and Best's extracts by subcutaneous route. However, on 20 December 1921 Joseph Gilchrist had already received pancreatic extracts by oral route. There is also a record in Banting's notes suggesting that, on 23 November 1921, Best had given to himself 5 cc of pancreatic extracts by subcutaneous route.

Collip, that was resentful for Banting and Best appropriation of his ideas, intended to independently register his own patent for the insulin extraction and purification method. After several discussions Banting, Best, MacLeod, and Collip agreed to establish a cooperation agreement and therefore avoiding an imminent break–up.[11]

The case report of Leonard Thompson was published in The Canadian Medical Association Journal. In the article Banting recognised the merit of the active ingredient purification to Collip: "one of us (J.B.C.) took up the problem of the isolation of the active principle of the gland".[45]

On 22 February 1922, Banting and Best published in the Journal of Laboratory and Clinical Medicine the article "The internal secretion of the pancreas" summarizing their animal experiments with pancreatic extracts.[40] The article had several inaccuracies. Lestradet identified 18 errors in the text and tables that, nowadays, would not be accepted by the editorial board.[13,36] One of the worst errors was the distorted citation of Paulescu's work. Banting and Best wrote: "He states that injections into peripheral veins produce no effect", but Paulescu wrote precisely the opposite: "Les mêmes effets, c'est–à–dire une diminution ou même une suppression passagère de l'hyperglycémie et de la glycosurie, s'observent aussi lorsqu'on injecte l'extrait pancréatique, non plus dans une veine périphérique, mais dans une branche de la veine porte…"[40,46] At this time, on 22 February 1922, seven patients with diabetes had already been treated with pancreatic extracts.[45]

On 25 February 1922 an agreement was made with the Connaught Anti–Toxin Laboratories, a non–profit organization, based on the campus of the University of Toronto, for producing insulin in Canada (Fig. 8).[F,47]

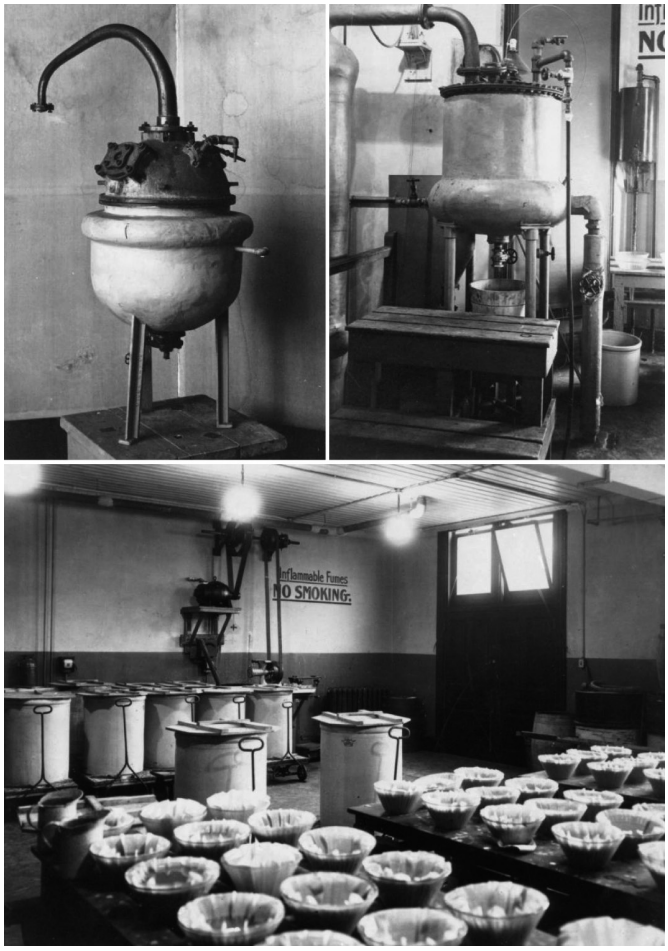

Figure 8. Connaught laboratory equipment used for purification and production of insulin in 1923.

Priority in the discovery of insulin and the award of the Nobel prize

On 3 May 1922, the results presented by MacLeod were received with great enthusiasm at the Washington meeting of the Association of the American Physicians.[30] Banting and Best had chosen not to attend the meeting saying that they could not afford the trip.[G]

Possibly, without knowledge of Meyer's work, MacLeod suggested the term *insulin* to designate the internal pancreatic secretion.[7,30,48,49] The team faced an ethical challenge: should or should not they patent their discovery? Banting did not want to associate his name with a patent. In fact, in the 18th and 19th centuries patenting by researchers and universities was discouraged.[50,51] The team and the University of Toronto eventually agreed to patent the discovery as a manoeuver to prevent anyone from securing a profitable monopoly and stalling the process of producing and distributing the extract to the patients.

On 18 September and on 9 October 1923 patents were registered by Banting, Best and Collip in Canada and in the United States, respectively.[52,53] On 19 December 1922, they sold for $1 the property of the insulin patent to the University of Toronto, which had created a special committee for controlling

[F] The Connaught Anti–Toxin Laboratories was later acquired by Sanofi–Aventis.

[G] The true reason is said to have been fury feelings against MacLeod because they feared he was appropriating their merit.[36]


CANADIAN PACIFIC RAILWAY COMPANY'S TELEGRAPH

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TELEGRAM

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DOCTOR FREDERICK G BANTING
INSTITUTE OF PHYSIOLOGY
TORONTOCANADA

THE THE ROYAL CAROLINE INSTITUTE HAS PRESENTED TO YOU TOGETHER WITH PROFESSOR W J R MACLEOD THE NOBELPRIZE OF THE YEAR 1923.

YOURS SINCERELY HJALMAR FORSSNER PRINCIPAL OF THE INSTITUTE

1030PM

Figure 9. Telegram announcing the Nobel Prize in Physiology or Medicine awarded to Banting and MacLeod in 1923.

insulin licensing, patenting and commercialization.[54] The Toronto group had not the proper resources to manufacture the extracts on a commercial scale. Taking in consideration the production problem and the humanitarian urgency of large scale distribution, the University of Toronto settled down an agreement with the pharmaceutical company Eli Lilly, granting for a year the exclusive right of producing and selling (at cost or gratis) insulin in the Americas, except in Canada, while any development and improvement on the production method, among other royalties, would be shared with University.[55] With the same humanitarian purpose, agreements with other 13 companies were settled between 1923 and 1925.[56]

In 1922, August Krogh (1874–1949) and his wife, Marie Krogh, both leading scientists in Denmark, travelled to the United States. The former was a respectful physiologist that had won the 1920 Nobel Prize in Physiology or Medicine on the regulation mechanism in the capillaries during exercise. August Krogh later exerted an influential role in the Nobel Prize nominations. Marie Krogh (1874–1943) was a physician that attended several patients with type 1 diabetes and herself had been diagnosed with diabetes the year before. MacLeod invited the Danish couple to visit Toronto.[57] During their stay, August Krogh secured an exclusive license for producing insulin in Scandinavia and, in 1923, in association with his wife's doctor, Hans Christian Hagedorn (1888–1971), Krogh founded the Nordisk Insulin Laboratorium, the embryo that later became the reputable NovoNordisk™.

According to the Karolinska Institute archives, the Nobel Prize nominations for the 1923 award were the following: Banting by George Washington Crile and August Krogh, and MacLeod by George Neil Stewart and August Krogh. A joint nomination of Banting and MacLeod was also proposed by Krogh.[58] August Krogh, who did not meet Best during his visit to Toronto, because he was out of town at that time, considered the young medical student of little importance to the discovery of insulin and therefore did not nominate him. In one of the fastest priority recognition of the Medical History, the Nobel Committee awarded Frederick Banting and John Macleod with the Nobel Prize for Medicine or Physiology in 1923 (Fig. 9).[59] Banting shared his prize with Best and MacLeod shared his prize with Collip.[60]

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