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History

Insulin: Discovery and Controversy

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During the first two decades of the 20th century, several investigators prepared extracts of pancreas that were often successful in lowering blood sugar and reducing glycosuria in test animals. However, they were unable to remove impurities, and toxic reactions prevented its use in humans with diabetes. In the spring of 1921, Frederick G. Banting, a young Ontario orthopedic surgeon, was given laboratory space by J.J.R. Macleod, the head of physiology at the University of Toronto, to investigate the function of the pancreatic islets. A student assistant, Charles Best, and an allotment of dogs were provided to test Banting's hypothesis that ligation of the pancreatic ducts before extraction of the pancreas, destroys the enzyme-secreting parts, whereas the islets of Langerhans, which were believed to produce an internal secretion regulating sugar metabolism, remained intact. He believed that earlier failures were attributable to the destructive action of trypsin. The name "insuline" had been introduced in 1909 for this hypothetic substance. Their experiments produced an extract of pancreas that reduced the hyperglycemia and glycosuria in dogs made diabetic by the removal of their pancreases. They next developed a procedure for extraction from the entire pancreas without the need for duct ligation. This extract, now made from whole beef pancreas, was successful for treating humans with diabetes. Facilitating their success was a development in clinical chemistry that allowed blood sugar to be frequently and accurately determined in small volumes of blood. Success with purification was largely the work of J.B. Collip. Yield and standardization were improved by cooperation with Eli Lilly and Company. When the Nobel Prize was awarded to Banting and Macleod for the discovery of insulin, it aggravated the contentious relationship that had developed between them during the course of the investigation. Banting was outraged that Macleod and not Best had been selected, and he briefly threatened to refuse the award. He immediately announced that he was giving one-half of his share of the prize money to Best and publicly acknowledged Best's contribution to the discovery of insulin. Macleod followed suit and gave one-half of his money award to Collip. Years later, the official history of the Nobel Committee admitted that Best should have been awarded a share of the prize.

Hailed as one of the most dramatic events in the history of the treatment of disease, the discovery of insulin at the University of Toronto in 1921–1922 extended the life-span of diabetic patients and made Fred Banting an international celebrity.

Changing Careers

Frederick Grant Banting (1891–1941) (Fig. 1) grew up on a small Ontario farm and was educated in local schools. He entered Victoria College, the divinity school of the University of Toronto, to study for the ministry, but after three semesters of arts courses and no premedical preparation, he transferred to the university's medical school in the fall of 1912. When his studies were interrupted by World War I, he and other students enlisted in the Royal Canadian Army Medical Corps in 1915, but were sent back to finish medical school in an accelerated 15-month program. They were graduated in December 1916 with a bachelor of medicine degree. Banting was sent overseas with the rank of captain. After serving as a medical officer in a Canadian hospital in England, he was transferred to France with a field ambulance unit. In September 1918, he was wounded and moved to a hospital in England. For valorous conduct during the Cambrai campaign, he was awarded the Military Cross by the British government.

He returned to Toronto in March 1919 and joined the staff of the Hospital for Sick Children as a resident in surgery. In July 1920, he opened a general surgical practice in London, Ontario, ~110 miles west of Toronto. When this was slow in developing, he took on a part-time position as demonstrator at the local medical school (now the University of Western Ontario). For nearly a year, he taught anatomy and physiology. Although hardly qualified to teach physiology, he convinced F.R. Miller, head of the department, that he could stay ahead of his assignments to the students. He also assisted Miller, a distin-

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Fig. 1. Frederick Grant Banting.

From *The Discovery of Insulin*. ©1982 by Michael Bliss, The University of Chicago Press.

guished neurophysiologist, with experiments on the electric stimulation of the cerebellar cortex of cats.

Anatomy of Diabetes

On October 30, 1920, in preparation for a lecture on the pancreas, Banting's attention was drawn to the lead article in his November issue of *Surgery, Gynecology and Obstetrics*, "The Relation of the Islets of Langerhans to Diabetes, With Special Reference to Cases of Pancreatic Lithiasis" by Moses Barron of the University of Minnesota *(1)*. While doing routine autopsies, Barron had come across a rare case of the formation of a pancreatic stone. Rarer still, the stone had completely obstructed the main pancreatic duct. Although all the acinar cells had disappeared through degeneration (atrophy), most of the islet cells had apparently survived intact. In reviewing the literature, Barron pointed out the similarity of these observations to those that occur when the pancreatic ducts were blocked experimentally by ligation. In 1901, L.V. Ssobolew (1876–1919) had shown that a ligature of the pancreatic ducts in rabbits, cats, and dogs leads to gradual atrophy and destruction of the enzyme-secreting acinar cells, whereas the islet cells remained intact for weeks, with no evidence of sugar in the urine (glycosuria) *(2)*. Thus, experimental and pathologic evidence reinforced the belief that the islets were the key to explaining diabetes.

The islets were first described by Paul Langerhans (1847–1888), a German medical student, in his dissertation in 1869, but he could not suggest any function for them. They were named after Langerhans by E. Laguesse *(3)* in 1893. The importance of the pancreas in carbohydrate metabolism had been known since experiments by Joseph Freiherr von Mering (1849–1908) and Oscar Minkowski (1858–1931) in 1890 *(4)*. They successfully removed the entire pancreas from a dog and observed all the symptoms of severe diabetes, namely, high blood sugar (hyperglycemia), glycosuria, and finally death involving ketosis and coma in 2 or 3 weeks. Their finding was the first experimental proof that diabetes may be of pancreatic origin.

In 1900, another piece of the puzzle was provided by Eugene Lindsay Opie (1873–1971), an instructor in pathology at Johns Hopkins, who described hyaline degeneration of the islands of Langerhans in cases of diabetes mellitus *(5)*. This discovery directed closer attention to the islets as the probable source of an internal secretion of the pancreas necessary for normal metabolism of sugar and lacking in diabetes. This was clearly inferred from the absence of glycosuria after ligation of the ducts and the atrophy of most of the gland. Apparently, the part of the gland that prevented the disease was the part that remained almost intact many weeks after ligation of the ducts. Islet tissue is also affected, but much more slowly than the acinar tissue.

In 1905, Ernest Henry Starling (1866–1927) coined the term "hormone" (Greek: *hormaein*, to set in motion) to designate the chemical messengers of the body's endocrine glands. As early as 1894, Sir Edward Albert Sharpey–Schäfer (1850–1935), who often stated that histology was in the service of physiology, suggested that on morphologic grounds the islet tissue might be responsible for the internal secretion by which the pancreas produced its effect on the blood sugar concentration. In 1913, in lectures at Stanford University, he suggested the name "insuline" for the still hypothetical substance in the islets *(6)*. He later acknowledged that he was unaware that the term had been introduced by Jean de Meyer (1878–1934) in 1909 *(7)*. "Insulin" (Latin: *insula*, island) was independently adopted by the Toronto workers in 1922.

The Big Idea

Banting suspected that in failed attempts by others, the active principle was probably destroyed by digestive enzymes in the acinar tissue of the pancreas during extraction of whole gland. That night, unable to sleep for thinking about the article and the lecture, Banting, at ~0200 in the morning, had an idea and wrote a note to himself. In what is considered his most authoritative statement on the discovery, the Cameron Lecture *(8)*,

delivered in Edinburgh in 1928, he recalled writing: "Ligate pancreatic ducts of dogs. Wait six to eight weeks for degeneration. Remove the residue and extract". In an unpublished 1940 memoir *(9)*, Banting gives a slightly different wording and time interval. In his notebook kept in the archives of the Academy of Medicine in Toronto, he actually wrote: "Diabetus. Ligate pancreatic ducts of dog. Keep dogs alive till acini degenerate leaving Islets. Try to isolate the internal secretion of these to relieve glycosurea" *(10)*.

In addition to the obvious misspelling of diabetes and glycosuria, the true notation does not contain the word "extract". In seeking to relieve glycosuria, he appears to be identifying diabetes with glucose in the urine in the traditional way, rather than following the newer concept of hyperglycemia as the identifying parameter of diabetes. Unfortunately, blood sugar analysis was difficult, required from 10 to 20 mL of blood, and was time-consuming and not very accurate. Furthermore, repeated withdrawal of such large volumes of blood could be harmful to human or animal. Consequently, it was more practical and safer to test the urine. Banting did not know about recent improvements in analysis of blood sugar on small volumes of blood, which would provide more frequent results that were clinically more useful and reliable than tests on urine, for measuring short-term fluctuations. These new sugar methods (discussed later in the text) were a very important development for diabetes research.

By ligating the ducts and allowing time for the degeneration of the acinar cells, Banting hoped to obtain the internal secretion of the islet cells free from the destructive action of trypsin and other pancreatic enzymes in the external secretion of the acinar cells. All of Banting's accounts of his inspiration and of the subsequent events were written years after his life had been changed by these events, and not everywhere did he record them the same. Consequently, they are not a reliable guide to the events in which he participated *(10)*.

Banting's conception was not new or altogether correct, because the digestive ferments of the pancreas must be activated in the intestine before they can exercise their destructive action. Many attempts to prepare extracts of the pancreas had been made by other investigators. Temporary sugar-reducing effects were often accompanied by harmful side-effects, such as fever and painful abscesses, which overshadowed any benefit. Georg Ludwig Zuelzer *(11)*, Ernest Lyman Scott *(12)*, Israel S. Kleiner *(13)*, John Murlin *(14)*, and Nicolas C. Paulesco *(15)* had all been able to produce pancreatic extracts that often reduced hyperglycemia or glycosuria in animals and, in some cases, in humans. However, toxic reactions after the initial relief of symptoms usually brought the tests to a halt.

A Cool Reception

F.R. Miller advised Banting to bring his idea to John James Rickard Macleod (1876–1935) (Fig. 2), professor of physiology and department head at the University of Toronto and a leading authority on carbohydrate metabolism, who was in a position to provide Banting with research facilities to test his proposal. Macleod had come to Toronto in 1918 after 15 years as professor at Western Reserve University in Cleveland, OH, and was president of the American Physiological Society during the year of discovery of insulin. In 1913 he had published *Diabetes: Its Pathological Physiology*. Although he concluded that there was an internal secretion of the pancreas, he believed it might never be separated in a pancreatic extract.

Fig. 2. John James Rickard Macleod.
Courtesy of the *Journal of Laboratory and Clinical Medicine*, Vol. 20, 1934–35.

The conventional wisdom during Macleod's training had been the primacy of the nervous system in the control of physiologic functions, with the liver having the central role in carbohydrate metabolism. Macleod's concept of diabetes was the traditional one of hyperglycemia and/or glycosuria, attributable to a failure of glycogen formation and storage in the liver producing an increase of blood glucose. His long-time interest was in trying to locate the center in the brain where the nervous control of carbohydrate metabolism, i.e., glycogenic function of the liver, originated *(16)*.

Only 4 months after arriving in London, Ontario, Banting visited Macleod for the first time on Monday, November 7, 1920. Macleod, knowledgeable about the literature, was skeptical; he found that Banting had only a superficial textbook knowledge of previous work on the effects of pancreatic extracts in diabetes and showed very little practical familiarity with methods for investigating such a problem. Macleod pointed out that many eminent scientists had tried to isolate this hormone and failed, without obtaining any conclusive evidence of the existence of an internal secretion *(17)*.

It is easy to understand why Macleod was not impressed with Banting or his idea. Banting was at a marked disadvantage in facing Macleod. He had no advanced degree, no honors, publications, experience in research, or teaching or private practice or surgery. Except for his work in the army, he had virtually no credentials. Lacking both training and expertise, Banting did not understand the advances made by others or the limitations of his own research proposal. Ignorant of the field and unsure of his methods and the chemical testing procedures he would need, Banting would require much help and direction *(16)*. He also failed to evaluate his own original erroneous assumption that the external and internal secretions are antagonistic within the pancreas. His weak background knowledge and inexperience in research kept him from a careful review of the literature for others who had searched for the internal secretion. He admitted on more than one occasion that had he been thoroughly acquainted with the literature before beginning the research, he might never have begun. "Too much reading of the literature is . . . inadvisable for [the] wide diversity of opinion and confusion of thought" *(18, 19)*.

Change of Mind

Banting met twice more with Macleod. Whether it was the persistence, the boundless enthusiasm, or the possibility of more reliable results with the new glucose methods on small volumes of blood, Macleod may have considered Banting's proposal worth thinking about. Finally, having cautioned Banting about the time it would take and the likelihood of negative results, Macleod agreed to provide laboratory space *(17)*. Now Banting became cautious. He did not want to give up his appointments in surgery and physiology in London to get negative results, even if they were of "great physiological value", as Macleod repeated several times. He told Macleod he would consider the whole matter carefully. Although there was nothing new in producing atrophy of the acinar tissues by duct ligation, there was interest in the relative degeneration of the acinar and islet cells. Nobody had tried to administer an extract from a fully degenerated pancreas. The great difficulty had been in getting ligation to work so that the pancreas atrophied. It was not until March 8, 1921, that Banting wrote to Macleod saying he would like to spend the second half of May plus June and July in Macleod's laboratory, if the offer of facilities was still open *(17)*. Whatever the experimental surgical techniques were to be, the results would be measured by tests of glucose in the blood and urine.

Heads or Tails

One day in May, Macleod introduced Banting to the two student assistants he had employed as demonstrators in the physiology laboratory. Charles Herbert Best (1899–1978) (Fig. 3), and Edward Clark Noble (1900–1978) were seniors in the physiology and biochemistry honors program and were planning to pursue a master of arts degree. They had also been working as research assistants in the production of experimental diabetes and had acquired experience in the analysis of blood sugar. Macleod also wanted measurements of the ratio of glucose (dextrose) to nitrogen (D:N ratio) in the urine. The D:N ratio was thought to be a particularly accurate reflection of the diabetic state. Macleod left it to Best and Noble to decide how they would divide the summer in helping Banting test his hypothesis. What began as a summer research job for a student turned into one of the most exciting and controversial medical adventures of modern times.

Fig. 3. Charles Herbert Best.
Courtesy of Eli Lilly and Company Archives.

A coin toss decided who would go first. Best won. Stevenson *(20)* cites Banting's Cameron Lecture *(8)* as the source of the story, but adds that Best and Noble dismissed it as a newspaper fiction. When this version was repeated by J.R. Henderson *(21)* in 1971, Noble was quick to respond that the coin toss did occur *(22, 23)*. When he did return he did not replace Best because Best was getting on so well with the experiments. There was no point in having a new assistant start fresh to pick up on the procedures at this stage of the work. Banting and Best had both become proficient with each other's techniques and worked well together. Noble did participate later in the post-discovery development work, and his name appears on several of the publications.

Best did all the chemical testing, measuring blood and urine sugar and urinary nitrogen, and assisted in other ways in the experiments on the depancreatized, duct-ligated, and normal dogs and in the preparation of active extracts. Blood sugar estimations were made by the Myers–Bailey *(24)* modification of the Lewis–Benedict *(25)* method. The results with this procedure were confirmed by the recently published Shaffer–Hartmann *(26)* iodometric titration method at high and low percentages of blood sugar. In the follow-up report on human subjects with diabetes mellitus *(27)*, blood sugar was estimated at intervals by the revised Folin–Wu *(28)* method, urine sugar by Benedict's *(29)* method, and acetone bodies by Van Slyke's methods.

Off and Running

Work began on May 17, 1921. The general pattern of the research was worked out with Macleod, who gave them suggestions about the surgical techniques, the preparation of chilled saline extracts of pancreas (he later suggested alcohol extraction) and helped them get started by assisting on the first dog. The widely held belief that Macleod set Banting and Best to work and then immediately left town for vacation in Scotland is not true. They had been at the research for almost 1 month, consulting with Macleod during this time. Macleod reviewed the status of the project, left his address, and gave parting instructions before leaving on June 14.

After ligation of the ducts, the dogs were expected to recover from the surgery and live more or less normally. After several weeks, the pancreas, unable to secrete fluid into the duodenum, would gradually atrophy and would be removed and processed to extract the internal secretion. The extract would then be administered to other dogs made diabetic by removal of the pancreas. It was a laborious task for some one with no experience in animal work, and it did not go well at first as Banting struggled to improve his surgical technique. By the end of the second week, 7 of their 10 dogs had died. To resupply the animal cages, they resorted to buying dogs on the streets of Toronto for $1.00 to $3.00 with no questions asked of the suppliers *(30, 31)*.

Because of surgical problems and the necessary passage of time before evaluation of the dogs after duct ligation, it was July 27 before both a depancreatized dog and a duct-tied dog were ready. On July 30, following Macleod's directions, they chopped a degenerated pancreas into small pieces and placed it in ice-cold Ringer's solution in a chilled mortar seated in freezing brine solution until the mixture partly froze. The mass was ground up with sand and a pestle, filtered, and warmed to body temperature. Five milliliters were administered intravenously to a dog whose pancreas had been removed. Samples of blood were taken at 0.5-h intervals. The dog's blood sugar fell from 0.200% to 0.120% in 1 h. The improvement was of short duration. Despite additional injections, the dog's blood sugar started to increase, and its death the next morning was probably hastened by infection *(32)*. It was their first experimental evidence that they had isolated an extract with antidiabetic principle.

The duo repeated their experiments and recorded frequent decreases in blood sugar and in sugar excreted in the urine on two additional depancreatized dogs. They named the extract "Isletin". Although the dogs died, Banting and Best were excited by what they had seen. "I have so much to tell you that I scarcely know where to begin", Banting wrote to Macleod on August 9. He added that the extract "invariably" causes a reduction in blood sugar, improves the clinical condition of the dog, and is destroyed by boiling and that extracts of other organs are inactive. They gradually eliminated possible sources of error by running control experiments. However, they also had many failures with dogs that died shortly after some of the surgeries.

A New Approach

As early as August 17, 1921, having run out of duct-tied dogs, they made an extract of a dog's whole fresh pancreas. A decrease in blood sugar from 0.300% to 0.170% was obtained 1 h after 10 mL of extract was administered *(32)*. On August 19, with the dog starting to weaken, they tried something different to avoid the external secretion with its toxic materials. They stimulated a pancreas with the hormone secretin until the pancreas was exhausted. This involved a complicated surgical procedure to obtain the crude secretin, followed by the slow injection of secretin for almost 4 h until the flow of pancreatic fluid through a cannula in the pancreatic duct stopped. The pancreas was quickly removed and processed to obtain the extract, which worked very well. Although exhausted gland extracts were not practical, they provided evidence supporting the goal of obtaining extracts of the islet cells free from the products of the acinous cells. Their work with duct-ligated dogs had been unnecessary, but they had gained knowledge without which they might never have developed the skill and insight to get the internal secretion from an easier source.

Clash of Personalities

When Macleod returned from vacation on September 21, he could hardly believe how much had been accomplished. He questioned the accuracy of their data. Banting resented this as a reflection on his integrity and could not restrain his natural tendency to be combative. His temper flared, and a bitter argument followed. Mutual friends interceded and the immediate storm blew over, but an atmosphere of friction settled in and clouded the background of their relationship and never entirely cleared. Macleod's aloof scorn of Banting's ability and Banting's growing bitterness at what he perceived to be Macleod's negative attitude kept relations always painfully strained *(33)*.

Macleod wanted them to repeat the summer's work to confirm their results before they proceeded with purification and assay. Macleod suggested an experiment to rule out dilution by the injections as causing the decrease in blood sugar. Banting used the meeting to request a salary, a separate room to work in, a laboratory boy to look after the dogs, and repairs to the floor of the operating room. Macleod was reluctant to provide these, believing that some other research would suffer. Banting threatened to leave and go to the Mayo Clinic or Rockefeller Institute. Macleod said he could go, but then relented and within a day or two after the confrontation Banting's requests were met. Macleod provided a salary and arranged for retroactive pay for Banting ($150) and Best ($170) in view of the decidedly satisfactory results of the summer. To provide further help to Banting, professor Velyien Henderson gave him an appointment in the department of pharmacology at $250 a month to fill a temporary vacancy. It was a great relief to Banting to have financial support for the winter months.

Banting, driven by conviction and passion, was eager for the work to advance more rapidly to testing humans with diabetes. He asked Macleod if J.B. Collip, a biochemist who was spending part of his sabbatical in the department of pathological chemistry, could join them. Collip had met Banting and learned about the insulin project shortly before Macleod left for Scotland. Macleod advised against expanding the team at this stage. He wanted Banting and Best to complete their independent research as originally planned. If the results continued to be satisfactory, Macleod would join them with his assistants *(18)*. So they went back to their dogs.

There Must Be a Better Way

Duct ligation and secretin exhaustion, although of great scientific interest, were strictly laboratory procedures and incapable of large-scale repetition to produce enough material for clinical use. On November 15, with only one duct-ligated dog on hand and faced with the built-in delay of this procedure, they realized that supply of extract was the bottleneck limitation of their project. There could never be a practical clinical application of the internal secretion of the pancreas unless they could come up with a better way of obtaining pancreatic extract.

In their reading they recalled that Laguesse had found that in the pancreas of fetal and newborn animals, islet cells were more abundant in relation to the acini than in the adult animal. Because there was no need for digestion until after birth, it was likely that there was little or no active acinar tissue in the fetus and that external secretion was absent or weak. Therefore, the fetal pancreas might be a practical source of an extract rich in internal secretion but free from the destructive enzymes of pancreatic juice. Although their focus was to avoid getting trypsin into the extract, they were also eliminating, to a considerable degree, the proteins other than insulin that were the real offenders causing toxic reactions *(20, 34, 35)*.

Having been born and raised on a farm and familiar with stock breeding, Banting remembered that cattle prepared for slaughter would first be impregnated to make them heavier eaters to hasten their fattening. There would always be a supply of fetal calves at the abettoirs to maintain an adequate supply of active principle for testing. Extracts of fetal pancreas worked well. Here too, however, although more productive than duct ligation or secretin exhaustion, it was obviously limited as a supplier of raw material. There was only one sufficient source—the pancreas of the adult animal.

Preliminary Communication

Macleod asked Banting and Best to present their research to university students and staff at the Physiological Journal Club in November. Best was to show charts of dogs, and Banting was to describe the work. However, in his opening remarks, Macleod said all the things Banting had planned to say about earlier research. Banting was inexperienced as a speaker, nervous, and not very articulate, especially after Macleod's surprising introduction. In writing about the meeting a year later, he noted how often Macleod was using "we" in describing the work. His state of mind was not helped when he learned that afterward, students were talking about the remarkable work of Professor Macleod *(36)*.

Banting and Best finished their first paper in late November. Macleod polished the final draft but declined being listed as a coauthor because it was Banting and Best's work *(17)*. The paper's title was "The Internal Secretion of the Pancreas" *(32)*, and it was to appear in the *Journal of Laboratory and Clinical Medicine* in February 1922. Before actual publication, there would be an occasion for the first public presentation before the American Physiological Society in New Haven, CT, on December 30, 1921. As a member of the society, Macleod's name was listed first on the program and Banting and Best were identified as "by invitation". On the published half-page abstract, the order was reversed *(37)*. Banting was nervous and "spoke haltingly, Macleod beautifully" *(38)*.

The leading investigators of diabetes were there. Two of them, Kleiner and Scott, had made extracts that reduced hyperglycemia and glycosuria. Participants asked many questions about the experiments, some of which were difficult for Banting to answer satisfactorily. It was obvious to Banting how badly he spoke and that he had failed to convince the audience that their results proved the presence of an internal secretion of the pancreas any more effectively than had previous investigators. Macleod came to his rescue by joining the discussion and trying to answer the friendly but serious criticisms. What especially bothered Banting was how smoothly Macleod had stepped in as though he owned the project, referring to "our work" and using the word "we" although he had never done a single experiment. Banting began to revisit all the previous negative interactions with Macleod. It was this intervention in the discussion that convinced Banting, who had never liked Macleod or felt at ease in his presence, that Macleod was trying to take over the project and steal his results and the fame. He began telling this to his friends *(9, 17, 36, 39)*.

Also in the audience was George H.A. Clowes, research director for Eli Lilly and Company. He recognized the potential of the research and asked whether his company could collaborate with the Toronto group in preparing the extract commercially. Macleod told him the work was not sufficiently advanced for commercial manufacture *(17)*.

Omitted from the published paper were the badly done experiments of the early summer. The paper contained minor factual errors. Figures in their graphs sometimes disagreed with figures in the text and/or, according to Bliss *(34)*, with figures in their laboratory notebooks. The clutter of data in their graphic displays reveals their inexperience in preparing papers for publication. Their statement that the extract always produced a decrease of the concentration of sugar in the blood and in excretion of sugar in the urine is not correct. Some extracts had not worked at all. Despite claiming that their extract contained the internal secretion of the pancreas, Banting and Best specifically said they did not yet have an agent that would "justify the therapeutic administration of degenerated gland extracts to cases of diabetes mellitus in the clinic" *(32)*. What was impressive about their experiments was the overall pattern of successful results.

Whole Pancreas

On December 6, they decided to use alcohol in preparation of a fetal calf extract. Macleod had suggested alcohol months earlier. Best recalled later that it had occurred to the three of them independently. Alcohol had been used by Zuelzer and Scott. The problem with an aqueous saline extract was that any attempt to concentrate it by boiling off the water also destroyed the active principle. Alcohol evaporates at a much lower temperature than water. They used a technique Macleod had shown them of a current of warm air flowing over the solution. Could they get a similar result from fresh adult pancreas? On December 11, whole pancreas was macerated and extracted with slightly acidic 95% alcohol, the filtrate was evaporated to dryness in a warm air current, and the dry residue was emulsified in Ringer's solution and given intravenously. The solution was injected into the dog whose pancreas they had removed. Its blood sugar dropped from 0.460% to 0.180% in 3 h. This was a major advance. Whole pancreas extracted with alcohol worked; there was no need for degenerated pancreas or fetal pancreas. Now the research could go ahead using cheap easily obtained fresh whole beef pancreas *(35)*.

Convinced of their success with diabetic dogs, Macleod finally agreed to help them develop their valuable discovery. He discontinued his own research on anoxemia and turned all the resources of his laboratory over to the new work. They needed help because the pace was speeding up now that they had the means for producing large amounts of extract. There was much to be done, and Banting wanted it done quickly so they could get to clinical testing. Macleod had now agreed to everything Banting had asked for, including his earlier request for Collip to join them.

Insulin Purified

In early December, Macleod invited James Bertram Collip (1892–1965) (Fig. 4), an experienced Canadian biochemist from the University of Alberta in Edmonton, to work on the task of purification. Collip was a Toronto alumnus of 1912 (BA) and PhD (1916), and had some knowledge of glandular secretions and the making of tissue extracts *(40)*. His sabbatical was supported by a Rockefeller Foundation Traveling Fellowship. Collip's laboratory was in the pathology building on the grounds of the hospital and several blocks from the dog quarters in the medical building. New research was now begun under Macleod's direction.

The pancreatic extract consisted of fats, proteins, water, salts, other organic materials, and the active principle. Different proteins are soluble at different concentrations of alcohol and different degrees of acidity. Banting and Best had discovered that the active principle from whole pancreas was soluble in ~50% concentration of alcohol. They discontinued fractionation at 65% alcohol because at this point the trypsin had been eliminated. Collip joined them at this stage. Applying standard experimental techniques to the problem, he started with fresh whole beef pancreas ground up in alcohol. After the mixture was filtered, Collip gradually increased the concentration of alcohol and found that the active principle remained in solution at progressively higher concentrations, whereas most of the proteins precipitated. The lipids and salts could eventually be removed by centrifugation and washing.

On the night of January 19, Collip discovered a limit; somewhere over 90% alcohol the active principle itself

Fig. 4. James Bertram Collip.

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was precipitated. Using this cutoff point he could remove most of the protein contaminants as a precipitate below 90% alcohol, then move to a higher concentration to precipitate and then isolate the active principle, as a powder, still with impurities but far purer than any previous extract. Collip tested the potency of the powders with methods he developed, using rabbits for the assay. After checking them for abscesses, he realized he had an extract sufficiently pure for testing on humans. Collip had found that pancreatic extracts were effective in lowering the blood sugar of healthy rabbits just as extracts had been in lowering blood sugar of diabetic dogs. This had great practical importance for it dispensed with the need to use depancreatized dogs for testing the potency of a batch of extract. This could now be done quickly and easily by testing the blood, easily obtainable from a vein in the rabbit's ear, and using the new micromethod of Shaffer–Hartmann. Clark Noble was added to the team to help with the rabbit testing. Macleod claims to have suggested using rabbits, which Collip then acted on *(17, 33, 34)*.

Clinical Trial Failure

In the winter of 1922, Best did the preliminary processing of the pancreas and making the initial concentration of material before handing it over to Collip for completion. By now Banting began to feel that he and Best were being brushed aside in the research. He became insistent that Macleod allow the first clinical test to be with an extract made by him and Best, for he was determined to participate in the first clinical trial. However, Banting, a surgeon not currently in practice, had no qualifications for experimenting on patients, and he had no standing at Toronto General Hospital, the university's teaching hospital across the street, where the trial would take place. He was not an expert clinician, and his limited postgraduate training had been surgical rather than medical. He had neither the knowledge nor the experience to take part on equal terms with his colleagues in the early clinical application of his discovery. On one side of the street he was no physiologist, no chemist; on the other side, he was no clinician. It was not an easy situation. Only a mature and well-balanced personality could have handled this state of affairs in good humor. Banting's personality was not mature. He was dynamic, forceful, impatient, and not always easy to get along with *(41)*. He applied for a temporary appointment in the department of medicine so he could test the pancreatic extract at the hospital, but was turned down. This only added to his sense of injustice. Macleod interceded with the head of the clinic to allow use of their preparation.

The extract, recalled several years later as "a murky, light-brown liquid containing much sediment, which dissolved to a considerable extent on being warmed" *(42)*, was administered by a young house physician on the afternoon of January 11, 1922. A total of 7.5 mL was injected into each buttock of the patient. Banting and Best waited in the hallway. They were not given samples of the urine because these were the property of the hospital. They would get the results the next day *(9, 36, 39)*. The patient was Leonard Thompson, a 14-year-old boy with severe diabetes who weighed only 65 pounds on admission on December 2, 1921. There was a drop in blood sugar from 0.440% to 0.320%, and the 24-h excretion of glucose fell from 91.5 g to 84 g. The Rothera test for ketone bodies continued to be strongly positive. No clinical benefit was observed *(27)*. A sterile abscess developed at the site of one of the injections, caused by the impurities in the extract. The extract was not effective enough to justify further administration. The record of the hospital indicated that Thompson received "Macleod's serum".

A reporter for the Toronto *Star* learned about the test and found his way to Macleod, who emphasized that the work was preliminary. The article appearing on January 14 emphasized Macleod's cautious responses. Banting was not pleased by the repetition of "we". Macleod, still unaware of Banting's suspicions, finally learned of his accusations of stealing his work and that he had been spreading these sentiments for some time. Banting's chief

complaints concerned the New Haven session and the *Star* interview as evidence of Macleod's bad faith. He produced confirmation of Macleod's domination of the New Haven meeting from others who were there. Although Banting's and Macleod's recollections of the involvement of a mutual friend trying to broker a peaceful settlement do not match, they agreed on a modus vivendi and effort at better communication. Macleod also agreed to continue the collaboration and said he "had no intention of robbing him of any of the glory that was his due". All those who participated in the researches on the physiologic action of pancreatic extracts would be listed on publications in alphabetical order. This placed Banting first and Best second *(17)*. While all this was going on, Collip, under pressure to come up with a better extract, was at work trying to produce a purified extract.

The Big Fight

In the winter of 1922, Banting considered every friend of Macleod's as his own sworn enemy and a biased partisan in the great Macleod conspiracy. One day in the latter part of January, after the less than satisfactory first clinical test and annoyed by Banting's attitude, Collip threatened to withdraw from all further cooperative experiments and start producing insulin independently. Collip told them he had solved the problem, was leaving the group, and intended to take out a patent in his own name on the purification of their pancreatic extract. He refused to tell them what the process was and added that Macleod had agreed that he should not tell them. This was a breach of an agreement between Collip, Banting, and Best to exchange all results. Banting, never short of righteous anger or noted for meekness or restraint when he felt wronged, exploded with clenched fists, and in a moment Collip was lying dazed on the floor of the laboratory *(43)*. Fortunately, he was not seriously hurt. There are no contemporary reports of this encounter, no reference by Collip, and only two accounts *(39)*, neither of which, according to Bliss, should be considered entirely reliable. One was by Banting in his unpublished 1940 memoir, the other by Best in a letter to Sir Henry Dale, dated February 22, 1954. They differ in details. There was another reference to what Collip said, written by Banting in 1922 and published in 1982 *(36)*, without any mention of a fight or confrontation. Paranoia, distrust, suspicion, and rivalry were out in the open. For years the story of the fight made the rounds of the insulin gossip mill, becoming distorted with every retelling. A twisted version that had Banting attack Collip in the university halls appeared in Banting's obituary in *Time* magazine of March 17, 1941.

Apparently the verbal exchange provoking the confrontation reached the people with an interest in the insulin project. They acted quickly to forestall any precipitous action detrimental to the cooperation of the Connaught Laboratories, established during the war to produce vaccines and antitoxins, to manufacture insulin on a large scale. In a memorandum dated January 25, 1922, Banting, Best, and Collip agreed not to exploit the process of preparing an extract of pancreas by seeking a patent or commercial collaboration. Macleod also signed. No modification in research policy was to be taken without preliminary consultation between Banting, Best, Collip, Macleod, and J.G. Fitzgerald, director of the Connaught Laboratories *(39)*.

Clinical Trial Success

Treatment of the 14-year-old diabetic youth resumed on January 23, 1922, this time with a purified extract made by Collip. Daily injections produced immediate improvement. His blood sugar dropped from 0.520% to 0.120% the next day, and his glucose excretion decreased from 71.1 g to 8.7 g. Acetone bodies disappeared from his urine, and he looked brighter, felt better, and became more active *(27)*. This was the first clearly successful clinical test of the internal secretion of the pancreas on a human diabetic.

In February, six more patients were treated, all with favorable results. A series of clinical studies followed that defined the biological effects of insulin and established guidelines for its clinical use. W.R. Campbell and A.A. Fletcher were the clinicians assigned to work out the many problems in utilizing this new therapy. A preliminary report was published in March under the title "Pancreatic Extracts in the Treatment of Diabetes Mellitus". The results of the clinical tests were described, with special emphasis on the first patient *(27)*.

The key sentence was clear: "These results taken together have been such as to leave no doubt that in these extracts we have a therapeutic measure of unquestionable value in the treatment of certain phases of the disease in man". The paper was sent to the *Canadian Medical Association Journal*, a publication with little circulation outside Canada, to assure quick publication. A more important presentation was scheduled in 7 weeks for a conference in Washington, DC.

Banting had little to do with the writing of this paper or the clinical work it reported and had no role in the ongoing experimental or clinical research. He and Best provided extract and depancreatized dogs and did other surgical work required for the experiments by the clinicians. They had to learn all they could, and quickly, about the substance they had and its clinical impact on diabetes, and they had to develop the production of much larger quantities of the extract. Macleod organized this ongoing research. Results were not discussed with them, nor were plans for future experiments. "Best and I became technicians under Macleod like the others", Banting wrote bitterly in 1940 *(9, 39)*.

Banting had been living and working under intense emotional stress. During March 1922, his attendance at the laboratory fell off. The only way he could overcome his despair at night was to drink himself to sleep, sometimes stealing the 95% alcohol from the laboratory. "I do not think there was one night during the month of March 1922, when I went to bed sober" *(9, 16, 39)*.

Despite the triumph of the research, his work and future were still uncertain. He sulked and raged and schemed for credit. Having started the work, he saw it taken over by others just when the good results started coming in. Banting's friends knew his state of mind. One of them, a physician and former teacher of Banting, alerted a *Star* reporter who was one of his patients to the work on the pancreatic extract. Through him the reporter met Banting and Best and prepared a long article to coincide with the publication of the *Canadian Medical Association Journal* on March 22. There were extensive quotes from the journal article and pictures of the four principals. However, Banting was interviewed at length, and the story was told very much as the work of Banting and Best. It was the first article to report the story from Banting's point of view.

Making It Official

In April 1922, the Toronto team prepared a paper summarizing all the work to date. The authors were Banting, Best, Collip, Campbell, Fletcher, Macleod, and E.C. Noble. For the first time they gave a name to the extract—insulin. They did not know that "insuline" had been proposed earlier. All the authors agreed that Macleod, who was a member of the host society, would present the paper at the Washington, DC meeting of the Association of American Physicians on May 3, 1922. The paper was titled "The Effect Produced on Diabetes by Extracts of Pancreas". Its presentation was greeted by an unprecedented standing show of thanks by the society, the first time in 20 years it expressed its appreciation in this way *(44)*. The discussion was printed as a supplement to the *Transactions of the Association of American Physicians (45)*

Thus, 2 weeks short of a year since Banting and Best began their work, the Toronto group, speaking through Macleod, announced to the medical world that they had discovered insulin and described its therapeutic success. Banting and Best did not go to Washington, the excuse being the expense of the trip. Macleod regretted not insisting on their attendance. Bliss believes that Banting, motivated by his continuing resentment of Macleod, decided to stay home and persuaded Best to do the same *(17)*.

Something Goes Wrong

Confident of its therapeutic value, the Toronto team made plans to manufacture insulin on a large scale, financed and administered by the Connaught Laboratories. Collip was to direct insulin production. Special equipment was installed in the basement of the medical building. Then disaster struck. To everyone's dismay and surprise, Collip, working with more variables than he was aware of, lost the knack of making insulin. As is frequently the case on passing from small- to large-scale production, great difficulties were encountered, so much so that for >2 months it was impossible to obtain extracts with anything like the potency of those used on the diabetic patients. The yield became unsatisfactory, at first, not in large batches, then not by any method. It was a gradual breakdown beginning sometime in March. A frantic struggle ensued during April and May as everyone pitched in to regain the key to making insulin. Although later accounts disagree on who did what, it turned out to be more than ever a team effort.

The problem and the solution lay in the heating of the extract during evaporation of the alcohol. Variations in the pressure of the water being supplied to the crude vacuum pumps were causing significant variations in temperature and, hence, in evaporation time. Somehow the heat neutralized the active principle. They replaced the vacuum stills with the earlier warm air current method of evaporation used by Banting and Best at Macleod's urging. By mid-May they had recovered the ability to make insulin. The modification involved using acetone with slight acidification. (Best several times claimed he was first to use acetone.) The pancreas–acetone mixture was filtered and then set in enamel-lined trays. An old exhaust fan supplied the wind. Heating coils above the trays heated the air as it passed over. After a 10-fold concentration, the temperature always below 35 °C, the rest of the process was Collip's method with alcohol *(17, 46–48)*. They later discovered that the pH was much more important for the solubility of the components than the temperature of the evaporation.

Enter Eli Lilly and Company

By mid-May enough insulin was being produced to permit resumption of limited clinical testing. However, attempts to produce the hormone in large quantities continued to fail. The team realized that they needed help. Toronto accepted an offer of collaboration from Eli Lilly and Co., and an agreement was worked out. Best and Collip traveled to Indianapolis to tell the Lilly chemists all they knew about making insulin and helped with the first attempt. The process worked. His appointment in Toronto ended, Collip returned to his position as professor of biochemistry at the University of Alberta.

Problems still remained. Every attempt to increase the yield of extract produced in Toronto failed, and the quality was not good. Protein impurities caused abscesses in many patients, and salts in the solution made many injections very painful.

Eli Lilly began work on insulin early in June. Their preparations from pork pancreases were potent from the beginning, but they too found it very difficult to increase the yield or achieve full strength in every lot. By August 19, 1922, Eli Lilly started shipments to the newly opened diabetes clinic at Toronto General Hospital. The Connaught facility had its special new vacuum apparatus and was about to start production. However, insulin still remained in short supply to the end of the year. Neither product, from Lilly or Toronto, had been purified or standardized. Lilly's lot-to-lot potency varied by 25%.

Clinicians had to be on constant guard for the symptoms of hypoglycemia from too much insulin.

Canadian production remained difficult, erratic, and expensive throughout 1922. Even with the new vacuum equipment, Connaught Laboratories still could not make enough insulin to supply Banting and other clinicians in the city. The Americans also had problems, and there were complaints in the autumn of 1922 about the lack of potency and rapid deterioration. Part of the difficulty was that Toronto and Lilly were using different-size rabbits in their tests for potency.

Precipitation at the Isoelectric Point

Attempts to prevent deterioration led Lilly's chief chemist, George Walden, to the company's great advance in insulin production and purification. He discovered that marked deterioration took place in the pH range 4.0–6.5. Whereas other sites producing insulin had, by luck or design, managed to avoid deterioration, Walden studied the process. He realized that the insulin solution was weakened by the gradual formation of a precipitate containing the active principle, thereby reducing the activity of the remaining solution. Insulin was being precipitated at the wrong pH. Walden also discovered that this precipitate was much purer and more potent than anything produced previously. Instead of avoiding the isoelectric point at which insulin precipitated, Walden now adjusted the solution to this pH to produce maximum precipitation. This yielded the best insulin yet, with a stability and purity from 10 to 100 times greater than any obtained before. The isoelectric precipitation method was developed between October and December 1922. The production problem solved, by February 1923 the company was building up large reserves of insulin. The one remaining problem was standardization. Consistency from batch to batch still varied by 10%.

A Patent for Protection

On January 23, 1923, an American patent on both insulin and Toronto's method of making it was awarded to Banting, Collip, and Best. For $1.00 to each, the three discoverers assigned their patent rights to the Board of Governors of the University of Toronto. The application had stressed that none of the other researchers in the past had been able to produce a nontoxic antidiabetic extract. A patent was necessary to restrict manufacture of insulin to reputable pharmaceutical houses who could guarantee the purity and potency of their products. It would also prevent unscrupulous drug manufacturers from making or patenting an impotent or weakened version of this potentially dangerous drug and calling it insulin.

Problems and disagreements about patents and licenses were eventually straightened out. Toronto needed a licensing arrangement that would make use of Lilly's resources for production without surrendering control of the extract. For a time there was concern about a possible challenge to a Lilly patent application for the isoelectric precipitation method from a team at Washington University in St. Louis that had made a similar discovery simultaneously and independently of Lilly. Phillip Shaffer and his associates Edward A. Doisy (1893–1986) and Michael Somogyi discovered the isoelectric precipitation method in the fall of 1922 and reported it in December 1922 *(49)*. After meeting with representatives from Toronto, Shaffer agreed to oppose the Lilly patent application if this would help Toronto. Armed with this agreement, Toronto was able to get Lilly to accept a new nonexclusive licensing contract. In return, Toronto dropped its objection to "Iletin", Lilly's trade name modified from Banting and Best's original term "Isletin", so long as "Insulin, Lilly" was given equal prominence on the company label.

"Get Used To It"

Who deserved the fame and tributes for the discovery of insulin? The press would have something to say about that by the way it covered the story. After the May 3 announcement, Banting, the country surgeon, and Best, the student, dissolved into the background and were replaced by Macleod and the clinicians. During the summer of 1922, the circumstances changed. Collip was gone, Best was working as director of Connaught's insulin production to help pay for medical school, and Macleod was doing research at the Marine Biological Station in St. Andrew's, New Brunswick, preparing extracts from islet tissue of a species of bony fishes (teleosts). These islet tissues are anatomically separate from the rest of the pancreatic tissue. It was a simple matter for Macleod to prepare extracts from the different tissue sources. Whereas the islet tissue was a potent source of insulin, none could be obtained from the pancreatic tissue proper. Thus, Macleod provided the first strong direct experimental evidence for the hypothesis that insulin is derived from the insular and not the acinar tissue of the pancreas *(50)*.

With Best, Collip, and Macleod away, Banting became the go-to man on the scene in the struggle to produce good insulin. He got to know Clowes and others in the scientific world outside of Toronto during this period when the clinical phase overshadowed the experimental physiology handled by Macleod.

Banting regained his self-confidence, but something new soon set him off again. There were more headlines, confrontations, and clashes in his ongoing war with Macleod. On September 6, the Toronto *Star* ran a story on the impact insulin was having overseas. It quoted a letter to *The Times* of London by Professor Sir William Bayliss of University College. Bayliss, codiscoverer with Starling of secretin, was one of Britain's leading physiologists and a friend of Macleod. He complained that Macleod was not getting proper credit for the duct-ligation method of producing pancreatic extracts, and he dismissed Banting as one of the collaborators who had possibly helped in the clinical application. Best showed the article to Macleod,

who denied having anything to do with the letter and told Best he did not want to get involved in a newspaper controversy by refuting it. Probably meaning to explain that every scientist had to learn to adapt to the attention and distortions in the press, Macleod said, "Banting will have to get used to it". Hearing this second hand, it sounded like Macleod was saying that Banting had better get used to all the credit going to Macleod. It was not long before Banting was in Macleod's office with the reporter who wrote the story, asking for a correction of Bayliss's statements. Macleod's response did not satisfy Banting or Best, nor did the follow-up story satisfy Macleod *(17, 36, 51)*.

Macleod's letters to Bayliss, Collip, and others during this period made passing references to "this fresh outbreak of Banting's", "an extremely uncomfortable position here", and "unbelievable trouble" and show how unpleasant the situation had become *(51)*. Macleod also complained that Banting wanted "full credit for all the work which has been done subsequent to this [ductligation] experiment. This I will of course not do since he has participated very little in the work, and not at all during the past six months" *(52)*. The modus vivendi worked out that spring had completely broken down. The old suspicions, misunderstandings, and distrust had reappeared. The controversy between Banting and Macleod was well underway. The discovery of insulin was up for grabs.

One Event—Three Versions

At this point entered Colonel Albert Gooderham, prominent member of the Board of Governors, patron of the Connaught Laboratories, and chairman of the Insulin Committee. Anxious to end the growing dispute, he decided to intervene. In September 1922, he asked Banting, Best, and Macleod to prepare their own understandings of the discovery of insulin. Each was asked to outline Collip's contribution. Gooderham did not write to Collip. He planned to compare the statements and then meet with them to clear up all misunderstandings and prepare one agreed-on history.

Macleod wrote the longest account and was quite certain that at every step he had given Banting and Best appropriate assistance, support, encouragement, and advice. He had criticized Banting's early proposals because Banting had come to him with such superficial knowledge. He had criticized the early results and demanded better to strengthen the credibility of the work. At first, he resisted premature clinical testing. To emphasize his belief that the young men should get full credit for their experiments, he had explicitly declined their offer to add his name to their first paper in the February 1922 *Journal of Laboratory and Clinical Medicine*. He had bent over backward not to claim as much as other research directors might have. "In many, if not most, laboratories it is the custom for the 'chief' to have his name on the papers when the investigation is in a subject related to that in which he is engaged and if he stands responsible for the conclusions and has participated to the extent that I did in the planning of the research. By this step I made it perfectly evident that I considered the full credit for this investigation to be Banting and Best's. This is surely what counts in questions of priority" *(17)*.

In his private and public accounts of the discovery of insulin, Macleod was very careful to credit Banting with having initiated the work and having confirmed the hypothesis that the pancreas contained an internal secretion. Macleod wanted Collip to get full credit for the purification of the extract and therefore played an important part in the success with the first diabetic human. The work was ultimately a team effort "working under my direction, of which Dr. Banting was one".

Banting, on the other hand, insisted that he alone had the idea that led to the discovery of insulin and that Macleod had been critical and discouraging of his work at every turn. Banting had no memory of any of Macleod's specific suggestions; what he did remember was that Macleod had not done a single experiment. According to Banting, Collip had joined the project only after the important advances had been made. He was willing to credit Macleod only with the investigation of insulin's physiologic action, but he and Best had discovered insulin well before that study was begun. In an appendix to his 1922 account, Banting listed several more examples of Macleod showing "a lack of trust and co-operation" *(36)*. This ungenerous, self-centered report reveals Banting's insecure personality and his fear of becoming sidelined.

Of the three accounts, Best's was the shortest, only ~1000 words, but perhaps the most objective. He gave much more credit to Macleod than Banting did, confirming that Macleod suggested the use of alcohol as an extraction agent. He also gave more credit to Collip than Banting did, although not on the important point of methods of purification. There was no review of injustices or any sense of grievance similar to those that permeated Banting's and Macleod's documents. "The work during the fall months reported in our two papers was performed entirely by Banting and myself. We had the benefit of Dr. Macleod's advice, but as he states, we were given the opportunity to conclusively prove the efficiency of our extract upon diabetic animals, and . . . diabetic patients, before the other members of the Physiological Staff participated in this work" *(36)*.

There was no reconciliation of the conflicting accounts, then or later. The same events were being described from different perspectives, with different emphasis, and different memories of events. There was no meeting with Gooderham, and no comprehensive account of the discovery of insulin was ever prepared at Toronto. The documents were not made public, and no more statements were made to the press.

Had Gooderham sought comments from Collip, he might have received something like the feelings he expressed in a letter to a friend. Probably written in 1923,

Collip said: "There are some people in Toronto who felt that I had no business to do physiological work. . . . The result was that when I made a definite discovery my confreres instead of being pleased were quite frankly provoked that I had had the good fortune to conceive the experiment and to carry it out. My own feelings now in the matter are that the whole research with its aftermath has been a disgusting business" *(53)*.

The Man From Copenhagen

Late in November, the University of Toronto had an important visitor. Professor August Krogh (1874–1949) of the University of Copenhagen, the most recent winner of the Nobel Prize in Physiology or Medicine in 1920 "for his discovery of the capillary motor regulatory mechanism" during exercise, had come to the US to deliver the Silliman Lecture at Yale. Everywhere he went he found American medical men talking about the insulin work at Toronto, so he decided to see for himself. He had a special interest in insulin because his wife suffered from diabetes mellitus. Macleod was delighted to welcome him and invited Krogh to be his house guest. Krogh was in the city November 23 and 24, observed the work, spent much of his time with Banting and Macleod, and gave a guest lecture on the capillaries. When he left, he had authorization from the University of Toronto to introduce insulin into Scandinavia. A nonprofit Nordisk Insulin Laboratory was in production by the end of 1923 *(54)*.

About Face

Apparently Macleod privately changed his appraisal of the discovery when it appeared that a Nobel Prize was a possibility. Two months after his response to Gooderham, he told the visiting August Krogh that Banting and Best would have gone off on the wrong track without his advice and guidance *(51)*. A copy of Macleod's original assessment was found among his papers 13 years after his death, and a copy was sent to the president of the University of Western Ontario in 1949. For the next 30 years, the Macleod manuscript was circulated among a small group of scholars. It was long impossible to gain access to the responses to Gooderham locked away in storage at the University of Toronto where they lay buried for >50 years *(55)*. Best had challenged the accuracy of Macleod's 1922 account and advised the president of the University of Toronto to forbid publication of any of the documents with the invalid claim that they were the property of the university *(56)*. Publication was suppressed by the university president to avoid reopening a controversy that he believed would do no one any good. However, the feeling by several eminent Canadian academic scientists was that Macleod had been roughly handled by history and the University of Toronto and deserved a chance to speak for himself. Although threatened with legal action if he searched for or quoted from Macleod's report, Lloyd Stevenson, a biographer of Banting (1947), published the document in the *Bulletin of the History of Medicine* in 1978 after Best died that year *(17, 55)*. Although there are no startling revelations, the details, nevertheless, differ markedly from the generally available versions. The 1922 account of the discovery of insulin by Banting, Best, and a list of contributions by Collip was published in the same journal in 1982 *(36)*.

Public Relations and Image

Relations among the principals at Toronto continued to be tense. Banting had always talked freely to his friends; they in turn talked to reporters, leaking details of Banting's hardships, difficulties, and other injustices done to him. Banting now hated Macleod with a passion, an attitude he never abandoned. There was a grudging persistence to Banting's dislikes. The depth and duration of his animosity long survived the general recognition of his own highly deserved rewards. Refusing for years to speak to Macleod, Banting was a lasting hater *(20)*. The most violent expression of his feelings was written in the 1940 memoir. Using terms like "grasping, selfish, deceptive, self-seeking and empty of truth . . . unscrupulous . . . a coward and a skulking weakling. . . . ", the memoir was laced with invective *(51)*.

According to people who knew Macleod personally, he was a gentle, honest, dedicated scientist, perhaps a little shy and reserved, perhaps a little vain, urbane, and cultivated. He was by temperament conservative, a cautious scientist, not brilliant or imaginative, but sound and plodding. He was respected for his organizational abilities, high standards of research, and skill in conveying ideas and information in a way that stimulated his students. They flatly denied that he was authoritarian on the German model. On the basis of these conversations and on Macleod's correspondence, Bliss believes that Macleod was contemptuous of Banting for his crude manners, dress, and language and his ignorance as a researcher *(51)*.

Wrong, Wrong, Wrong

Scathing criticism of Banting and Best's work as reported in their first two publications in the *Journal of Laboratory and Clinical Medicine* came in a letter to the *British Medical Journal* in December 1922 from Dr. Ff. Roberts, a physiology researcher in Cambridge, England. The writer declared that because the proteolytic enzyme exists in the pancreas in an inactive form—trypsinogen—that is activated by enterokinase secreted by the small intestine, there was no physiologic basis for the duct-ligation experiment. Although trypsinogen is also activated when a pancreas is cut out and begins to deteriorate, this happens only slowly and can easily be prevented by chilling. "The production of insulin originated in a wrongly conceived, wrongly conducted, and wrongly interpreted series of experiments. Through gross misreading of these experiments . . . apparently beneficial results have been obtained in certain cases of human diabetes. . . . The exper-

iments of Banting and Best show conclusively that trypsin *qua* ferment has nothing whatever to do with it" *(57)*.

Roberts was immediately rebuked for the harsh tone of his letter by Henry Hallett Dale (1875–1968), a leading figure in British research, who shared the Nobel Prize with Otto Loewi (1873–1961) in 1936 for discoveries relating to the chemical transmission of nerve impulses. Dale had visited Toronto in September 1922 and been favorably impressed by Best's work on insulin production and by his potential as a scientist. Dale called the review "armchair criticism" that leads only to verbal controversy. Perhaps "the enthusiasm, which carried them further, was fired by an imperfect interpretation of their earlier results. . . . Nobody can deny that a discovery of first-rate importance has been made, and, if it proves to have resulted from a stumble into the right road, where it crossed the course laid down by a faulty conception, surely the case is not unique in the history of science. . . . it is a poor thing to attempt belittlement of a great achievement by scornful exposure of errors in its inception" *(58)*.

Banting and Best were not experienced and knowledgeable enough to have achieved success without input and other help from an experienced investigator like Macleod. The immediate chilling of the pancreatic material, as suggested by Macleod, stopped self-digestion of the fresh pancreas by the activated enzymes. According to Michael Bliss, "Banting and Best's research was so badly done that, without the help of Macleod and Collip, . . . the two young Canadians would be fated to disappear from medical history" *(56)*. Although duct ligation played no essential part in the discovery and was not the way to go, it set the stage for making extracts directly from the whole pancreas. So many of these were potent that it convinced Macleod that there really was an internal secretion, and he added new resources and additional staff to the project. In addition, the newly developed micromethods of sugar analysis made it possible to track the effectiveness of the extract in test animals by rapid and frequent blood analysis, a more reliable indicator than urine analysis. Unfortunately, smooth interaction between the players was difficult because of the insecurity and volatility of Banting's personality.

Fame, Celebrity, Recognition, Reward

Banting achieved a sudden and spectacular fame. The entire scientific world joined to hail him with lavish praise. He was made an honorary member of most of the major scientific and medical societies of the world. Other honors, prizes, medals, and awards pursued him in rapid succession. To the public he was the laboratory wizard from whom new miracles were expected hourly. Newspapers and magazines trumpeted his fame. Banting appeared on the cover of *Time* magazine for August 27, 1923, in the expectation by the editors that he was the logical choice for a Nobel Prize later that year. Tributes came from the prime minister and the opposition leader. On a trip to England he was received by King George V.

Banting was shy, unsophisticated, an ordinary country boy who hated speeches, banquets, and formal dress, and he hated being interviewed, to the point of being rude and insulting to reporters. He was at first an indifferent speaker with a stumbling delivery whose ineptitude seemed all the more noticeable when he was preceded or followed by the excellent delivery of Macleod. With practice, his public speaking improved and he became more business-like than eloquent.

Ever more suspicious of a Macleod conspiracy to deprive him of his well-deserved credit and recognition, Banting stayed in close touch with friends and well-wishers who were trying to advance his interests. Many of his friends thought that recognition should entail something more tangible than applause, luncheons, and memberships in exclusive clubs. There were discussions in the House of Commons in Ottawa and the provincial legislature of Ontario in Toronto about financial support to Banting and Best for their research. As the prospect of national honors for Banting developed, there was a rush of activity by his politically connected friends to provide a government grant. Letters were written to the leading American clinicians and others, including Charles Evans Hughes (1862–1948), US Secretary of State at the time and later reappointed to the US Supreme Court as Chief Justice, whose daughter Elizabeth had been treated by Banting, soliciting testimonials on behalf of an honor for Banting and insulin. Best was all but completely ignored. The university people did not consider him a codiscoverer. To them he was a student assistant. Banting often gave Best a great deal of credit and told the Premier of Ontario that he and Best had worked together from the beginning and that his and Best's names should be linked. However, at no time did he credit Best with specific ideas or proposals that advanced the research. The testimonials had their effect. Early in May 1923, the Ontario government announced the Banting and Best Chair of Medical Research, a nonteaching professorship for Banting. An annual grant of $10000 would pay his salary, support his research, and fund Best in his research. A special appropriation of $10000 would reimburse the discoverers for the discovery period. Banting gave Best $2500. On June 27, 1923, the Canadian House of Commons granted Banting a lifetime annuity of $7500. They had no way of knowing that of the four principals of the insulin team, only Banting would fail to make new discoveries.

The Big Prize, a Challenge, and Protests

When archives of the Nobel Committee in Stockholm were opened to historians for study of the 1923 awards, the documents revealed the process and roadblocks in the pathway leading to the prize in physiology or medicine *(53, 59)*. Early in 1922, the Caroline Institute's Nobel Committee sent out its annual requests for nominations of individuals worthy of receiving a prize for the discovery in physiology or medicine that, in that year, had, according to the will of Alfred Nobel (1833–1896), "conferred the

greatest benefit on mankind" *(53, 59)*. After discarding the self-nominations, publicity seekers, the frivolous, and the irrelevant, there remained a total of 57 nominations with merit. The prize could be awarded to more than one, but no more than three. Banting was nominated, so was Macleod. There was also a joint nomination of Banting and Macleod. It came from August Krogh, who had visited Toronto in November 1922 and heard the inside story from Macleod about the guidance he had provided to Banting and Best. Krogh nominated the pair for the discovery of insulin and their exploration of its clinical and physiologic characteristics. "According to the information I personally obtained in Toronto, . . . credit for the idea for the work that led to the discovery unquestionably goes to Dr. Banting. He is a young and apparently very talented man. But he would surely never have been able to carry out the experiments on his own, which from the beginning and at all stages were directed by Professor Macleod. The other authors should be considered as Macleod's and Banting's collaborators, but there is reason for specially mentioning the chemist J.B. Collip. He has made a very important contribution in the method of producing insulin. . . . But I do not think that is sufficient ground for the award of a prize". Krogh concluded that Macleod's part in the work merited the award. The Nobel Committee got the message.

In April 1923, the list was reduced to nine, counting Macleod and Banting as one. Nobel nominations are subjected to reviews. These appraisals are detailed, expert studies of the work of the nominees. Those assigned to the committees read the publications, observed the results of clinical tests, and met with specialists who were using insulin. On the basis of past experience, 1 year was almost always too soon to evaluate the true importance of a fundamental medical discovery. In their lengthy report the examiners concluded that the discovery of insulin was of fundamental importance, worthy of a Nobel Prize, and, although one of the reviewers found it difficult to judge Macleod's contribution, they agreed that Banting and Macleod should share the Nobel Prize. The recommendations had to go to the Nobel Assembly, which consisted of the faculty members of the Caroline Institute, for final approval. At its October 11 meeting, there was a challenge to the joint recommendation and it was sent back to the committee for reconsideration. The objection was to making an award on hearsay evidence from unknown persons or on statements in the two appraisals, like "it is beyond doubt", or on things that are thought of as "very possible". The Assembly should adhere only to verifiable facts.

The committee reconsidered and reaffirmed its recommendation. In a formal letter to the Assembly, it identified August Krogh as the source of the "hearsay" evidence and emphasized that he had made the joint recommendation based on his visit to Toronto. The committee concluded that it was Banting's idea alone, but "it was Macleod's guiding hand that helped Banting's idea reach such a happy culmination . . . ." On October 25, the 19 professors of the Caroline Institute voted by secret ballot to award the 1923 prize to Banting and Macleod "for the discovery of insulin". For once, as stipulated in Nobel's will, the award was given for a benefit rendered "during the preceding year". The citation made no mention of insulin's clinical or physiologic characteristics as noted in Krogh's nomination.

Banting was furious when he learned that Macleod was to share the Nobel Prize and said he would not accept the award. Gooderham, who knew the whole story, told Banting he must think of his obligations to Canada and science. How would it look if the first Canadian to receive this honor turned it down because of a difference of opinion about the Prize? Banting changed his mind. He decided to share the money award and the credit with Best. Macleod was on a ship returning from England when he heard the news. A few days after landing in Montreal, he telegraphed Collip and asked him to share his half of the prize money. Collip accepted. Macleod told the press "it is teamwork that did it". Banting and Macleod were each awarded an honorary Doctor of Science degree by the University of Toronto on November 26. Macleod was elected a Fellow of the Royal Society of London in 1923. Banting had to wait until 1935 for this honor.

The Nobel Committee received furious letters of protest from Georg Zuelzer in Berlin, pleading for some recognition of his priority. Nicolas Paulesco in Bucharest was outraged. He claimed his work was stolen by Toronto, and he demanded justice from the Nobel Committee. Scott *(60)* called attention to his prior successful experiments. The protests were ignored. Israel S. Kleiner (1885–1966), an American biochemist whose work in 1919, almost completely forgotten, was closer to success than any of them, made no claims *(61, 62)*.

Kleiner had made solutions of ground fresh dog pancreas in slightly salted distilled water. In all 16 of his experiments, using the new methods for blood sugar analysis *(24, 25)*, the extract caused a temporary but marked decrease in the blood sugar of depancreatized dogs, sometimes by >50%. There were also marked decreases in the excretion of sugar. There were, however, mild toxic symptoms, usually an elevated temperature, associated with the extract. This work "indicates a possible therapeutic application to human beings" *(13)*.

Making it to the Screen—Big and Small

From the very beginning, controversy over the discovery and the 1923 prize led to distortions in the popular perceptions, especially of Macleod's image. Banting, Best, and Collip had their ardent advocates. Macleod, however, remained a shadowy figure in the background of the story. He has been seen as intruding on the glory of Banting and Best and stealing some of their credit.

A 1973 British film, "Comets Among the Stars", later released as a three-part TV series, starred Sir Ralph Richardson in the role of Professor Macleod. His por-

trayal, as described by Stevenson *(55)*, was dark, unappealing, and repellent. All the villainous elements of conflict and drama were exploited. In 1988, a Canadian TV miniseries from Gemstone Productions, based on *The Discovery of Insulin*, made it to the small screen as "Glory Enough for All". It was seen in the US on "Masterpiece Theater" of the Public Broadcasting System in 1989 and 1990.

After Insulin

Macleod left the University of Toronto in 1928 to become Regius Professor of Physiology at his alma mater, the University of Aberdeen, where he later became dean of the medical school. When a farewell dinner was held for Macleod at Toronto, Banting refused to attend. Most of Macleod's Aberdeen days were spent in nagging pain from crippling arthritis. Macleod's textbook, *Physiology and Biochemistry in Modern Medicine* (1918), with collaborators reached a 7th edition in 1934. He died in 1935 at age 59. Macleod's replacement as professor of physiology at Toronto was 29-year-old Charles Best, who had graduated from the medical school in 1925.

During the years after insulin, Banting was coauthor on publications dealing with a wide variety of subjects, but he did no work with insulin *(63)*. It was indicative of his limitations as an original researcher that all the other members of the team except Banting went on to do other significant work. He tried to duplicate the insulin experience—a great idea, an ingenious approach, and then the solution. In his many talks on medical research, he always stressed the ideas, not the training. Throughout his life, the press and public expected him to repeat the triumph of insulin and were always asking what he would do next.

During the 1930s, he became close friends with Collip. When a Conservative government resumed accepting titles for Canadians in 1934, after suspension in 1919, Banting was made a Knight Commander of the British Empire by King George V. He was now Sir Frederick Banting, K.B.E.

A Fatal Plane Crash

When war broke out in 1939, Banting served as coordinating chairman of Canada's wartime medical research focusing on aviation medicine, especially the physiologic effects of the high speeds, high altitudes, and rapid descents expected to be encountered in combat aerial maneuvers. While in London in the winter of 1939–1940, consulting with his British counterparts, he spent his free time writing a long account of the discovery of insulin, which was deposited among the unpublished Banting Papers in the archives of the University of Toronto. Bliss describes the account as rambling and unpolished, never verified for accuracy—more a documentary source than a history. The 1940 manuscript was frequently cited in his history of the discovery of insulin. Bliss rejects the conventional history that minimizes Macleod's role in the discovery of insulin and reveals the importance of his contributions leading to the successful first use of insulin on human diabetics.

Banting returned to Canada in the spring of 1940. Early in 1941, he decided on another transatlantic trip, by air to save time. On the night of February 20, he was a passenger in a new Hudson bomber being ferried from Gander Bay, Newfoundland, to England. Shortly after takeoff one engine failed, and while returning to base, the other engine faltered and the radio failed. It was dark and snowing. Unable to see the ground, the pilot came within a few feet of a safe landing, but a wing struck a large tree at the edge of a frozen lake and the plane crashed. Only the pilot survived the crash. Banting sustained severe injuries and died ~20 h later. The remote crash site was spotted by a search plane on the 24th, and the victims were removed by sled *(64, 65)*.

To his colleagues of the insulin era, Banting was determined, willful, and frequently difficult. To others, he was "a disappointed and disillusioned man, . . . an unsociable creature. . . . Not a great scientist, as scientifically trained people appreciate the word, he was primarily . . . a symbol of medical research". Understood by too few, Banting was a man of many talents, moods, and interests. Immortalized long before his death, he was "a man possessed of the finest degree of humility. . . . " *(66)*.

In an obituary tribute, Collip wrote: "Banting was a most unselfish individual. He was always mindful of helping others and it was almost a religion with him to encourage, stimulate and assist young research workers" *(67)*.

A Mistake Has Been Made

Best and Collip went on to productive careers in research. Best, his associates, and students conducted basic studies on the lipotropic effects of the dietary factor choline and pioneered in the isolation and development of heparin. In 1941 he succeeded Banting as head of the Banting and Best Department of Medical Research. Banting's friends were extremely upset when Banting's chair and control of the department were given to Best. They knew that in the last years of his life, Banting had developed an intense dislike of Best, a feeling shared by E.C. Noble, who was deeply embittered by Banting's and then Best's neglect of his contributions to the insulin work *(56)*.

With Norman B. Taylor, Best coauthored a widely used textbook, *The Physiological Basis of Medical Practice* (1937), (10th edition, 1979). The 11th (1985) and 12th (1991) editions were edited by John B. West. Best wrote and often reminisced about his role in the discovery of insulin. However, his memory was too selective to make the accounts entirely reliable *(68)*.

After Banting's death, Best became the chief spokesman for the view that he and Banting had discovered insulin on their own in 1921 and had been denied their full share of the glory because of the scheming of Macleod and Collip and their friends. During the next 30 years, Best and his friends promoted a version of the discovery

of insulin with a greatly enlarged and enhanced part for Best while minimizing or omitting the contributions of Macleod and Collip. He justified revisions of the written record on the grounds that memory took precedence. Bliss believes that Best was insecure and had a deep psychologic need for recognition and a place in history *(56)*.

Best's version began to lose credibility with the surfacing of new documentary evidence of the vital contributions of Collip and Macleod. As already noted, Best challenged the accuracy of Macleod's 1922 account and urged the university president to forbid its publication. Collip refused to offer his own written comments or to get involved in the web of misleading claims, distortions, manipulation of the historical record, omissions, and inaccuracies being put out by Best. He was satisfied to let history have the final say. Best's rewrite of history was challenged in 1954 by a major critical evaluation of the insulin work. Joseph H. Pratt, a Boston physician, credited Collip with providing the first insulin to be used successfully in the treatment of diabetes. He concluded that all four members of the team deserved recognition *(69)*.

Best was elected a Fellow of the Royal Society of London in 1938 and, in the later years of his life, was awarded many honors by grateful diabetes associations, medical societies, and universities. He received the DSc in 1928 for postgraduate work in London with H.H. Dale. In 1950, Dale nominated Best for a Nobel Prize for his later research on choline and for his general achievements, including the work with insulin. Despite repeated nominations by Dale and others *(56)*, Best was not awarded a Nobel Prize. However, he did have the satisfaction of knowing that the 1972 official history of the Nobel Prize acknowledged that a mistake had been made in 1923. "Although it would have been right to include Best among the prize-winners, this was not formally possible, since no one had nominated him—a circumstance which probably gave the Committee a wrong impression of the importance of Best's share in the discovery" *(70)*. The history noted that "The work was also facilitated by the previous introduction of convenient methods for determining the sugar content of the blood". Years earlier, on the occasion of the 25th anniversary of the discovery of insulin, Best said: "We had many advantages over our predecessors, but I think the greatest single advantage undoubtedly was the method of doing blood-sugars quickly and accurately . . . on very small amounts of blood" *(71)*.

In 1981, Rolf Luft, a former chairman of the Nobel selection committee for the physiology or medicine award, told the NIH that in his view, the 1923 award to Banting and Macleod was the worst error of commission *(72)*. It was a message Luft had delivered before. At a 1972 anniversary symposium on insulin, he dismissed Macleod as a manager and promoter who "put Collip and the Lilly Company into business" *(61)*.

More Hormones, More Isolation

Collip did intensive pioneering work on isolation of the parathyroid hormone and added a DSc degree in 1924 and a MD degree in 1926. During 5 years in Edmonton, he related the hormonal control of calcium and phosphorus metabolism to an active principle in the parathyroid gland. In connection with this work, he needed a method for serum calcium that would be as simple as possible and at the same time give consistent results. The method of Kramer and Tisdall *(73)*, modified slightly by Tisdall *(74)*, was modified again, slightly, by Clark and Collip *(75)* in 1925. Their version for serum calcium was widely adopted in clinical chemistry laboratories for the next 40 years.

In 1927, Collip became chairman of biochemistry at McGill University. There his group of students and collaborators engaged in the forefront of wide-ranging endocrinologic research in the isolation and study of the ovarian, gonadotropic, and adrenocorticotropic hormones. Collip had the remarkable skill to handle large concentrations of glands, purify them to a manageable scale, and separate out different hormone fractions. He received many honors for his pioneering investigations in endocrinology and was elected a Fellow of the Royal Society of London in 1933. In 1947, Collip became dean of medicine at the University of Western Ontario.

In his obituary tribute to Banting, Collip wrote: "The part which I was able to contribute subsequently to the work of the team was only that which any well-trained biochemist could be expected to contribute, and was indeed very trivial by comparison with Banting's contribution" *(67)*. Collip was very reluctant to talk about the discovery of insulin. Very little of his unpublished material, including his laboratory notebooks, has been found. He did write a short history of the discovery of insulin, which he read at a medical meeting *(76)*. He always maintained that the truth about the discovery of insulin was in the publications of those years and might emerge after they were all dead *(56)*.

Footnotes

Insulin kept type I diabetic patients alive so that complications that occur later in life (cardiovascular, renal, blindness) were then better understood and appreciated. There was an unexpected by-product to the discovery of insulin. Diabetic individuals lived longer and passed the hereditary component of this disease to their children, which has brought about a steady increase in the number of diabetic sufferers.

In 1926, John Jacob Abel (1857–1938) of Johns Hopkins University prepared the first crystalline insulin. In the mid-1950s the molecular structure of insulin was determined by Frederick Sanger (1918–1982), for which he received a Nobel Prize for Chemistry in 1958. He received a second Nobel Prize in 1980 for determining base sequences of nucleic acids. With genetic engineering it is now possible not only to make insulin in unlimited

quantities, but to make human insulin rather than use the slightly different insulin of other species.

Summary

In retrospect, Banting, Best, Macleod, and Collip all made significant contributions, and Macleod's role was greater than what he has been credited with by the conventional history. Despite professional rivalry and personality conflicts, they showed that the missing factor in diabetic patients is in the islets of Langerhans and that a material can be extracted and purified from the islets that greatly extends the life of type I diabetic patients. A key player in their success was the new methodology of sugar analysis using small volumes of blood, which made frequent determinations possible in small animals and patients.

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