The Discovery of Insulin

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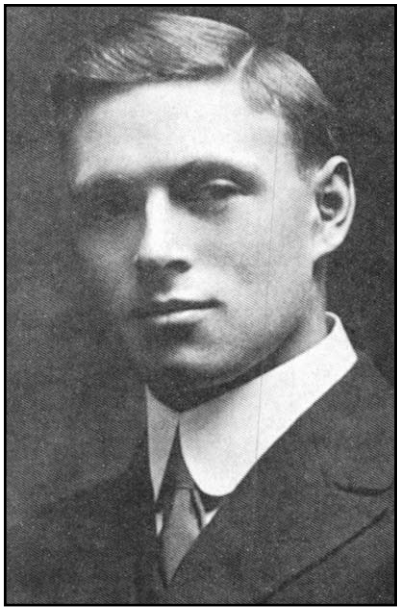
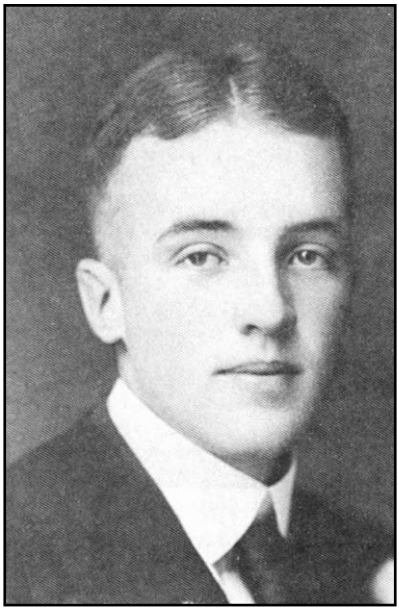
Frederick Grant Banting
(1891-1941)

John James Rickard Macleod
(1876-1935)



James Bertram Collip
(1892-1965)



Charles Herbert Best
(1899-1978)

Foreword

The discovery of Insulin, in Toronto, Canada in 1921-22, was one of the great medical breakthroughs of all time. Prior to this, Insulin Dependent Diabetes had been a universally fatal disease. In celebrating this discovery, we remind ourselves what life was like for patients before this time and we are encouraged to look to the future with hope that further breakthroughs will bring about the final defeat of this still potent and watchful enemy.

I have been involved in the treatment of diabetes for more than 20 years and have had the good fortune to have worked in the city where the discovery was made. This monograph is a tribute to the researchers past and present who have dedicated their lives to the understanding of diabetes and to the patients who have to contend with this disease daily.

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Diabetes Mellitus is a disease that has been known to physicians since descriptions of medical disorders were first recorded. Obvious references to diabetes appear in Sanskrit writings of the 6th century BCE as well as in ancient Egyptian papyrus. The classical description of diabetes was in the second century by the Greek physician Areteus of the Imperial Roman province of Cappadocia, in what is now Turkey. He described diabetes as being "a melting down of the flesh and limbs into the urine" and recorded the terrible way in which the patients succumbed to their illness. The famous Persian physician known to the West as Avicenna, writing at about 1000 CE, had divided diabetes into two groups with patients being either thin, younger, or more obese, older persons. Avicenna was perhaps the greatest medical genius in history who published the "Canon of Medicine", an encyclopedia summarizing classical and contemporary medical knowledge up to that time and which served as the standard text book of medicine for 500 years in both the Eastern Islamic and Western Christian spheres of influence.

By the Enlightenment it had been discovered that the urine of diabetes was sweet; with the substance being sugar, and dietary modifications were advocated. The benefit of a diet low in calories in patients with maturity onset diabetes was reinforced in 1870 during the siege of Paris by the Prussian forces during the Franco-Prussian War. The citizens of Paris were reduced to eating whatever they could find and it was noted that the prevalence of diabetes declined with this enforced weight reduction diet.

There the matter rested until 1889 when a German physiologist Oscar Minkowski visited his senior colleague Professor Von Mering at the University of Strassburg to discuss a shared interest in the problems of digestion. To further investigate the role of the pancreas they performed a pancreatectomy on a dog. This dog was tethered inside since no cage was available and, despite being house trained, was noted to pass copious amounts of urine on the floor. The urine was found to be loaded with sugar and the dog to be diabetic. Removal of the pancreas caused diabetes.

This advance in the understanding of diabetes mellitus occurred at the start

of the era of "organo therapy". Charles Brown-Sequard had ground up rooster testes and injected them into himself in order to restore the juvenile male vigor – so important to the French. He claimed good results from this though these were not reliably reproduced. There was no doubt however about the next advance in organo therapy when Dr George Murray, London England, used extract of thyroid to cure myxedema. Myxedema is the clinical condition found in patients who have a severely underactive thyroid. This treatment was an undoubted success and though this occurred in 1891, well over 100 years ago, medical treatment has never done better: successfully treating a potentially fatal disorder with a natural substance which has no side effects and is active by mouth.

For the next thirty years investigators attempted to emulate this success by using extract of pancreas to treat diabetes. Unfortunately results always followed the same pattern. Patients developed sterile abscesses at the site of injections and had severe toxic reactions with fever, malaise, and other symptoms which may have included hypoglycemia (low blood glucose), which went unrecognized at the time.

The toxic reactions the researchers faced were due to the pancreas in mammals having two roles. Most of the pancreas functions as an exocrine (Greek: external excretion) gland and delivers digestive enzymes by a duct into the gastrointestinal tract. Scattered throughout the pancreas are clumps of cells known as Islets of Langerhans which are the endocrine (Greek: internal secretion) part of the gland. Their function is to produce insulin and deliver it directly into the blood stream. It was the insulin which was sought to treat diabetes, but, crude extracts of the pancreas contained both digestive enzymes and insulin and it was the digestive enzymes which caused the problems.

The next major advance was by Dr Frederick Allen, an American physician in the early part of the 20th century. The previous animal model for insulin dependent diabetes had been the dog in which the whole pancreas had been removed. This however led to ketoacidosis with the animal dying in a day or two. Allen performed a 90% pancreatectomy in which case the

dog became diabetic but did not die immediately and was a much more realistic model of insulin dependent diabetes in the human. He found that by feeding these dogs a low calorie diet he was able to prolong their life significantly. This was translated into human clinical practice with patients being treated with the Allen diet which was essentially a starvation diet. This did succeed in prolonging patient's lives sometimes for years before the inevitable end due to starvation, ketoacidosis, or infection.

Work on extract of pancreas continued. Dr Georg Zeulzer, a Berlin researcher, even got as far as registering a patent in the United States for a pancreatic extract said to be suitable for the treatment of diabetes. It suffered the same problems as previous extracts, being thought too toxic for clinical use. In 1914, however, World War I broke out and Dr Zeulzer was called to the colors.

By 1918 the war had been progressing for four years. The Germans had made their last major attack in early 1918 using troops taken from the Eastern front after they had forced the Treaty of Brest-Litovsk on the Russians and had broken through British lines. They were stopped outside Amiens by five Australian divisions. In August 1918 the Allies counter attacked and started pushing the Germans back towards the borders of Germany. In this fighting the front line was often confused causing difficulties in evacuating casualties, and medical staff in the regimental aid posts were often involved in the thick of the fighting. Involved too was a medical captain with the Royal Canadian Army Medical Corps, Dr Frederick Banting. He was awarded the Military Cross for gallantry under fire and, in September 1918, was wounded in the right arm by shrapnel and spent the end of the war in hospital. He was repatriated to Canada and when he left the army decided to go into private practice as a surgeon. There were, however, quite a few surgeons being demobilized and he looked around for somewhere to set up his practice. He selected London, Ontario, a city about 100kms west of Toronto. Unfortunately, he had no contacts there, and referrals were few and far between. To fill in time he decided to take a lecturing job with the University of Western Ontario in London.

As one of his duties, one day, he was required to give a lecture on carbohydrate metabolism. Like any self respecting surgeon he knew nothing at all about carbohydrate metabolism and took some literature to bed with him to brush up. Dr Banting had what would probably be described now as Post Traumatic Stress Disorder and was quite an agitated, nervous sort of person and often found it difficult to sleep. One of the papers he read was by a Dr Moses Barron describing the outcome in a patient in whom a stone had lodged in the pancreatic duct and caused atrophy of the exocrine part of the pancreas. This gave Banting an idea and he lay awake for hours thinking it over. If he could tie off the pancreatic duct in an experimental animal, such as a dog, he might be able to destroy the part of the pancreas which produced the troublesome digestive enzymes and leave the Islets of Langerhans, which produce insulin, intact. If he were then able to obtain an extract of the pancreas it would not contain the digestive enzymes and when injected it would "relieve glycosuria" without the toxic problems encountered in the past.

He took this idea to his professor at the University of Western Ontario who referred him on to Professor John James Macleod of the University of Toronto.

Professor Macleod was an internationally famous researcher into carbohydrate metabolism and thought that Banting's idea had some merit. He felt that even negative results would be useful though did point out that these sort of ideas had been tried for thirty years previously without success. He arranged for Banting to be given space at the University of Toronto Medical School and some dogs so that work could commence during the Summer vacation. Professor Macleod then went to spend this time back in his home, Scotland.

Banting had as his assistant Charles Best, a physiology student who was spending a research term with Professor Macleod. Charles Best had also volunteered for the army and got as far as the United Kingdom as a sergeant of artillery before the war ended. He had tossed a coin with another physiology research student, Clark Noble, to see who would work

with Banting. It is said he won the toss, but Robert Volpe, Professor of Medicine at Wellesley Hospital in Toronto where I spent several years and who knew Charles Best for quite a long time, insists that Best lost the toss. It was thought better not to work with the irritable and melancholic Banting. Noble regretted the decision not to work with Banting for the rest of his life, although his name did appear on the first papers written by the group.

Banting's idea was to have several dogs. With the first dog he operated to ligate the pancreatic duct and waited for pancreatic atrophy. He then harvested the pancreas, ground it up, filtered it, and then used this extract on another dog, which had previously had its pancreas removed (pancreatectomised), and was thus diabetic, to see if he could reduce the dog's blood glucose and urinary ketone levels. He found that, although this extract did undoubtedly decrease the blood sugar in the dog, he ran into the same problems as had been encountered in the previous three decades, namely sterile abscesses, and general toxic reactions. Some dogs seemed to do a little better for a while before dying, others contracted infections, and some dogs seemed not to benefit at all.

Banting and Best continued working through the summer and autumn and into the winter with their dogs. They soon found however that there was no real advantage to the laborious procedure of tying off the pancreatic duct in the dogs to obtain the endocrine product, and that extracts taken from beef pancreas from the abattoirs worked just as well. Towards the end of 1921 they had started using alcohol to assist in extraction of the active component of their pancreatic extract. Professor Macleod was quite impressed with progress and suggested that a Professor Collip might be able to help. Professor James Bertram Collip, (known as Bert), Foundation Professor of Physiology from the University of Alberta, in Edmonton, was spending a sabbatical year with Professor Macleod and was an expert in tissue extraction. Collip introduced several modifications. He started using normal rabbits to test pancreatic extract by observing the fall of blood sugar, a lot simpler and quicker test system which did away with the need for pancreatectomised dogs.

In December 1921, Banting and Macleod journeyed to the American Physiological Society Meeting at Yale University in New Haven to present the results of their work so far. Banting made the actual presentation, but, throughout his life, he was a nervous, uncertain speaker and his paper did not go over very well. He was subjected to vigorous questioning from the floor along the lines that this avenue of research had been tried before. Professor Macleod, who was actually the Chairman of the session, and who was a very gifted speaker, came to the defense of his junior colleague. In the audience also was Dr G. Clowes, Research Director of Eli Lilly, a US drug company, who was sufficiently impressed to seek the couple out after the presentation and offer the support of his company in the research. Also present was Dr Elliott Joslin, founder of the famous Joslin Clinic, who was similarly impressed.

On the train back to Toronto, however, Banting was very distressed and sat up all night smoking, thinking over the day's events. He was quite disgusted at his own poor performance at his first major scientific presentation but he was also quite upset at Professor Macleod who had liberally used the word "we" when speaking from the Chair. Banting felt Macleod was not entitled to adopt such a proprietorial role since he had not taken an active part in the research project, and, in fact had been overseas during much of the work. Banting was also quite concerned that, with Professor Collip on the project, he was in danger of being overshadowed.

On return to Toronto, perhaps in order to establish precedence in the field, Banting prevailed upon Professor Macleod to try one of their more active pancreatic extracts on a human diabetic patient. Such a person was 14-year old Leonard Thompson who was an inpatient at the Toronto Hospital for Sick Children at the time. He was given 7.5mls of extract into each buttock and had the usual response. His blood sugar did fall, but, he developed a severe toxic reaction and sterile abscesses at the sites of injection. The trial was thought a failure.

Shortly after this, at the beginning of 1922, Collip announced to Banting that he thought he had cracked the problem of obtaining an active and

non-toxic extract. When Banting inquired as to how it had been done, Collip declined to tell him, perhaps thinking of future patents. Banting was outraged and grabbed Collip by the lapels and had to be restrained from further attacking him. Best was later to draw a cartoon entitled "The Discovery of Insulin" showing Banting sitting on Collip, with his hands around his throat, choking him. It must be added, however that Banting and Collip were later reconciled and became firm friends.

Leonard Thompson was readmitted to hospital and given an injection of Collip's new preparation which was now called Insulin to differentiate it from Banting's previous extracts which had been known as Isletin. This time the preparation worked like magic with no toxic side effects and the blood sugars fell remarkably. Unfortunately, Collip then forgot how he had actually isolated the active component. The long-suffering Leonard Thompson was discharged on no treatment apart from his Allen diet. Collip was later to describe himself as an intuitive "bath tub chemist" and in those days research notes were very sketchily kept. There followed a frantic several weeks as Collip, with the help of Eli Lilly, tried to rediscover the secrets of the process. Fortunately the procedure was soon worked out again.

What Collip had done was to extract the insulin into an acid alcohol water mixture relying on the fact that beef (unlike pork) insulin is soluble in acid solution. The alcohol was then evaporated off and the insulin precipitated by two to three molar saline. The resulting salt cake was then washed and re-dissolved in an acid solution giving the active component.

On the eve of the victory Banting now became quite depressed. He thought his work had been overshadowed by that of Collip and he started drinking heavily. There was prohibition of the consumption of alcohol in Canada at the time and the only way alcohol could be obtained was with a doctor's prescription. This was not a major problem for Banting who also had access to the laboratory alcohol used in insulin extraction. He announced to Best that he was quitting the group. Best however replied that if Banting left, he, Best, would as well and after some discussion Banting recollected his

strength and returned to the project. He did so at a fortunate time since there was now a problem as to who should give the insulin. Professor Macleod was Head of a very busy research department and did not have time to see the multitude of patients who now were clamoring for insulin. Professor Collip and Charles Best were not medical practitioners and were unable to give insulin and Banting now had literally nothing else to do. Professor Macleod started referring all his diabetic patients to Banting whom he referred to as "my clinical associate".

One of the first patients to receive the new insulin preparation was Elizabeth Hughes. She was the fifteen year old daughter of the American Secretary of State, Charles Hughes, who had run, in 1916, as the unsuccessful Republican contender against Woodrow Wilson. He was a very wealthy and powerful man, and sent Elizabeth with her private nurse to Toronto to virtually camp on the doorstep until insulin became available. She was to describe the miraculous transformation of her health as being "unspeakably wonderful". She survived until 1981, and in fact I was working in Toronto when her death created something of a stir in the academic department.

Elliott Joslin came up to Toronto, as did Frederick Allen, to see for themselves what was happening and were able to take the news back to their wards which were full of skeletal patients waiting for just such a breakthrough. Joslin was of New England Protestant stock and was well versed in the Bible. He was to write that the change in the patients reminded him of Ezekiel Chapter 37 which he referred to as the "Banting chapter".

> *"The hand of the Lord was upon me and carried me in the spirit of the Lord and set me down in the midst of the valley which was full of bones… and lo they were very dry.*
>
> *And he said unto me, Son of man, can these bones live? And I answered, O Lord God though knowest.*

Again he said unto me, prophesy upon these bones, and say unto them, O ye dry bones, hear the word of the Lord…

So I prophesied as I was commanded: and as I prophesied, there was a noise, and behold a shaking, and the bones came together, bone to bone.

And when I beheld, lo, the sinews and the flesh came up upon them, and the skin covered them above: but there was no breath in them.

Then he said unto me, prophesy unto the wind, prophesy, Son of man and say to the wind thus sayeth the Lord God; come from the four winds, O breath, and breathe upon these slain that they may live.

So I prophesied as He commanded me, and the breath came into them, and they lived, and stood up upon their feet, an exceeding great army."

Everywhere Banting went he was received with a standing ovation. So was Best. In fact, in 1978, fifty-six years later, I was attending the British Diabetic Association Meeting in London when an old man came into the front of the lecture theatre and was greeted with loud clapping. It was Charles Best attending the meeting only a few months before his death.

In 1923 Banting and Macleod were awarded the Nobel Prize, partly on the enthusiastic recommendation of Joslin. Banting was a bit unhappy about dividing the prize with Macleod and announced that he would share his with Best. Macleod shared his with Collip. An Act of Parliament was passed in Canada making Banting a permanent professor of medical research and founding an institute, the Banting and Best Institute, for him. Unfortunately Banting was not a trained medical researcher, and though over the next few years he tried various other research ventures, nothing much came of them. Such was his fame, however, that even noted researchers such as Hans Krebs spent several months with him in the late '30s.

In 1934 Banting was knighted and made a Fellow of the Royal Society. With the outbreak of World War II, Sir Frederick Banting put his research institute at the disposal of the Empire and his Institute did do pioneering work in aviation and high altitude medicine. In February 1941 it was arranged for him to fly to the United Kingdom to exchange defense research findings. This was to have been one of the first winter crossings of the Atlantic. He took off from Newfoundland in a Lockheed Hudson bomber but the carburetors froze up, resulting in engine failure, and the pilot made a crash landing. I understand from ex-RAAF pilots at Greenslopes Private Hospital that there is a certain critical altitude at which the carburetors will freeze and the correct procedure is to climb or dive above or below this layer. The pilot, however, was inexperienced in winter conditions, but did make a skillful landing, though the aircraft hit a rock hidden in the snow. There were no seatbelts for passengers in military aircraft in those days and when the pilot, who had hit his head on the instrument panel and temporarily lost consciousness, came to, he found that his three passengers, of whom Banting was one, were dead or unconscious in the aircraft cabin. The pilot administered what first aid procedures he could before making off to a distant farmhouse to summon help. When he returned he found that Banting had regained consciousness and had managed to open the aircraft door and had then fallen out into a snow drift where he succumbed to a combination of hypothermia and the results of his injuries.

Thus died Sir Frederick Banting, Nobel Prize winner, Fellow of the Royal Society, holder of the Military Cross and the most famous Canadian of his day, perhaps of all time.

Charles Best, meanwhile, went on to complete doctorates in both medicine and physiology. Later he was appointed Professor of Physiology at the University of Toronto, where he joined with Banting as a Director of the Banting and Best Institute. Among other achievements, he succeeded in isolating heparin, the widely used anticoagulant.

Let us return to the early '20s, just after the discovery of Insulin. Touring North America at the time was Augustus Krogh and his wife Maria. Krogh

was an internationally famous Danish physiologist who already had been awarded the Nobel Prize and now he was approached by the Nobel committee to give recommendations as to who should be recipients for the Prize for this great discovery. It was largely on his advice that the Prize was awarded as it was. Krogh had a particular interest in the problem since his wife Maria suffered from maturity onset diabetes. On returning home he approached Augustus Krongstedt, the owner of a Danish drug company, Leo Pharmaceuticals, to produce insulin in Denmark. Krongstedt enlisted the aid of a bright young medical researcher, Hans Christian Hagedorn.

The patent for producing beef insulin had been given to Collip and Best, the two non-medical members of the team, since it was felt inappropriate for the medical staff to be involved in commercial transactions. They transferred the patent to the University of Toronto who then licensed it to many places around the world. Leo Pharmaceuticals, with the assistance of Hagedorn, started to produce insulin, thus beginning the Danish predominance in insulin research and manufacture which was later to lead to the founding of Novo Nordisc. Eli Lilly produced the insulin in the United States under prior arrangements, using Banting's old name of "Isletin", and in Australia beef insulin was produced by Commonwealth Serum Laboratories. In Germany, insulin was licensed to the giant chemical combine I G Farben (Interessen Gemeineschaft Farben Industrie or the Consortium of Dye Manufacturers).

After World War II, the combine was broken up by the Allies into Baden Anilin und Soda Fabrik (BASF) which now produces Isoptin and Capsaicin Cream, Bayer which produces the Glucometer range of meters and Hoescht, producers of Daonil.

Research, however, had not stopped on diabetes during the war especially in Denmark, an occupied neutral country. In 1946 Hans Christian Hagedorn announced a new longer lasting insulin. When insulin had been first isolated, it had had quite a prolonged duration of action, but as it had been purified successively, the duration of action became shorter and shorter. It was realized that a protein was being extracted from the isolate

and an effort was made to find a protein that could be reintroduced to again make the insulin preparation longer lasting without being allergenic. Such a protein was Protamine, a four KDalton protein found in fish sperm. When combined with beef insulin and a small amount of zinc it forms long crystals which slowly dissociate giving an eight-hour insulin known as Neutral Protamine Hagedorn (NPH). In 1956 the same organization led by Schlichtkrull and Hallas Moller produced the Lente range of insulin by adding zinc to the insulin. Lente insulin produces crystals which are cuboidal blocks and result in a 12-hour time of action; however, these tend to line up in regular groups causing jamming of insulin delivery devices.

In 1958 insulin conferred another Nobel Prize when Frederick Sanger was awarded it for sequencing the amino acid structure of insulin: this being the first protein to be so characterized. In the 1960's and '70s we saw the introduction of a purified mono-component insulin and the changeover from beef to pork insulin which differs from human insulin by only one rather than three amino acids. In the 1980's human insulin itself was produced using recombinant DNA technology with E.coli or baker's yeast (Saccharomyces cerevisiae) being used to produce insulin in tanks, thus bypassing the need to harvest the increasingly more scarce pig pancreas'. The latest innovation is the introduction of synthetic insulin produced by substitution of various amino acids in the protein chains resulting in insulin of varying durations of action.

I find the story of the discovery of insulin to be very inspirational: though it has elements of intrigue and jealousy, it is very much the story of an ordinary and not particularly gifted man, Frederick Banting, who through persistence and dedication and despite personal problems achieved wonderful things. I am sure in the future we will see further marvelous breakthroughs in the management of diabetes, but, I doubt that any will affect the lives of diabetic patients as much as did the events that occurred in Toronto 80 years ago.

Acknowledgments and References

The details of "The Discovery of Insulin" are taken from two excellent books and a lecture by Professor Michael Bliss. These and other references are listed below.

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Charles Best (left) with Dr Frederick Banting (right) at the Medical School, Toronto, 1921.

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