

Historical perspective

From pancreatic extracts to artificial pancreas: history, science and controversies about the discovery of the pancreatic antidiabetic hormone

III. Purification of the pancreatic extract: John James Rickard Macleod, Professor of Physiology and James Bertrand Collip, Professor of Biochemistry



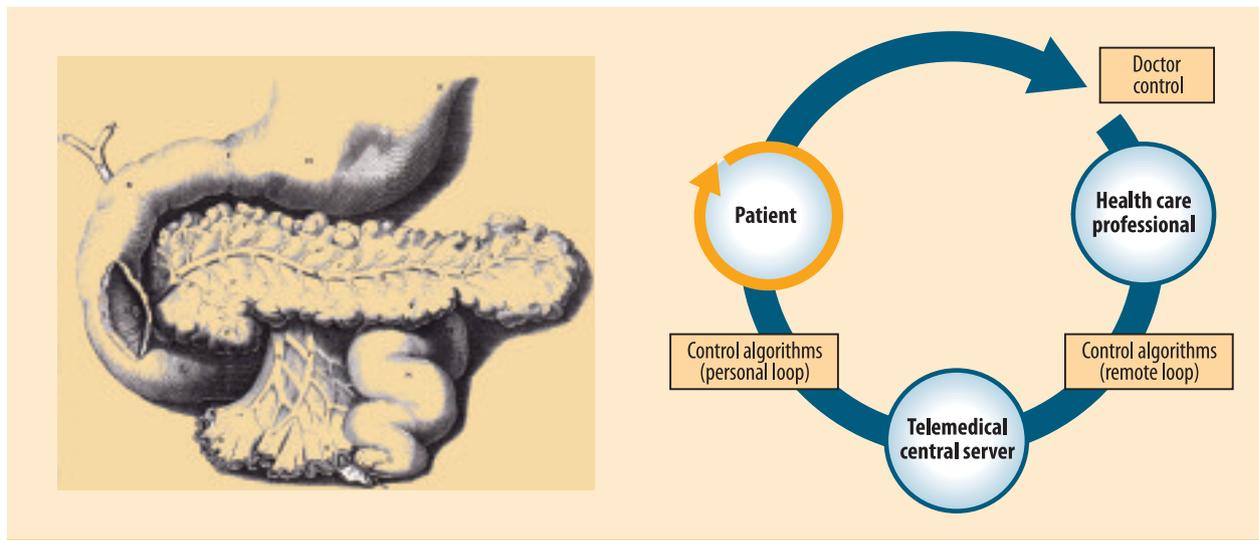
J.J.R. Macleod



J.B. Collip

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J.J.R. Macleod (1876-1935) Biographical Sketch

James Bertrand Collip wrote the obituary of JJR Macleod, published in 1935 by the Biochemical Journal,¹ providing the most relevant aspects

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of his biography. Born in Cluny, a little Scottish village, and son of the late Rev. Robert Macleod, was raised in Aberdeen, and graduated in Medicine with honors at its University (1898). Awarded by the Anderson Travelling Scholarship, Macleod spent one year at the Physiological Institute of the University of Leipzig, investigating the phosphorous content of muscle. He returned to work at the London Hospital Medical Col-

lege, becoming Lecturer in Biochemistry, and elected Scholar of the Royal Society. In 1903, at the age of 27 years, he married a second cousin, Mary Watson McWalter, and accepted the position of Professor of Physiology at Western Reserve University, Cleveland, Ohio, USA.

Macleod's initial research activities were devoted to intracranial circulation and caisson disease. At the West-

ern Reserve University he published on carbamates and purine metabolism. Then, he became interested in liver glycogen breakdown by different mechanisms (pique, splachnic stimulation, injection of adrenaline, and others), responsible of accelerating glycogenolysis. During his last years, he investigated the production of lactic acid by the muscle.

Between 1907 and 1914, Macleod published 12 full papers in the *American Journal of Physiology*, mainly focused in extending the observations of Claude Bernard on the nervous control of hepatic glycogenolysis.² In 1913, he published the monograph “Diabetes: its Pathological Physiology”, in which he pointed out that multiple attempts to reduced blood glucose by injection of pancreatic extracts had been unsuccessful³. Nominated Professor and Chair of the Department of Physiology, University of Toronto, Ontario, Canada, in 1918, he became an international reference in the field of carbohydrate metabolism. The offer of FC Banting and Charles H Best to demonstrate the reduction of hyperglycemia and glycosuria of depancreatised dogs after the administration of crude extracts of “degenerated pancreas” or fetal pancreas, triggered the return of Macleod to investigate various aspects of carbohydrate metabolism. His personal positioning regarding the respiratory quotient as made by two components, hepatic gluconeogenesis and peripheral carbohydrate oxidation, rejecting the constancy of the urinary dextrose-nitrogen ratio in the fasting diabetic animal, generated conflicts with many physiologists. In 1923, he received the Nobel Prize for Medicine, jointly with his collaborator, Frederick Banting. Then, he became elected Fellow of the Royal

Society in 1923 and was awarded with the Cameron Prize of Edinburgh University. After receiving the Nobel Prize, Macleod published many papers regarding the actions of insulin, including the biological consequences of the administration of particular fish pancreatic islets extracts to rabbits. He received honorary degrees from Toronto, Western Reserve, Aberdeen, and Jefferson Medical College in Philadelphia. Finally, he accepted the Chair of Physiology at the University of Aberdeen, in 1928.

Bitter discussions with Banting, who never accepted the relevance of the advice and scientific background of his mentor and professor, precipitated the decision of leaving Toronto in 1928 to become Regius Professor of Physiology at Aberdeen University. In his book, *The Discovery of Insulin*, published in 1983, Michael Bliss reinvinciated the reputation of J.J.R. Macleod, damaged by the accusations of stealing credits of activities made by his collaborators⁴ and the multiple attempts of Best to show himself as the major contributor to the discovery of insulin.⁵ Opposite to Banting and Best, Macleod and Collip, owners of a higher intellectual baggage, always depicted a modest and dignified behaviour.

In the last period of his career, Macleod investigated the production of lactic acid by the muscles and collaborated with researchers of the Rowett Institute on the rate of intestinal absorption of various sugars by the intestine. In association with Donhoffer, he also revisited the role of the nervous system in the glyco-genic activity of the liver, showing the potential role of the parasympathetic innervation in the stimulation of the hepatic gluconeogenesis. The

last textbook he wrote, *Physiology and Biochemistry in Modern Medicine*, was a great success.

Suffering a progressive rheumatoid arthritis, complicated with pleurisy and pericarditis, his health declined. He died on Saturday March 16th 1935, at the age of 58. He did not have children.

A final remark of Collip respect to the character of Macleod was: “It was typical of him that he will not allow an enthusiastic colleague or assistant to embark on an investigation without pointing out all the technical or theoretical difficulties which will probably be encountered, and yet would supply encouragement and all the practical assistance at his disposal”. This was, as an example, the case of his personal interactions with Frederick G. Banting.

Macleod’s main research achievements on carbohydrate metabolism before the clinical use of insulin
Glycogenolysis and the relation of the Nervous System to Carbohydrate Metabolism

Macleod was very much influenced by the observations of Claude Bernard on glycogenolysis, leading to the proposal that a centre situated at the medulla, closely related to the vasomotor centre, regulated the production of glucose from glycogen and the concentration of blood sugar (figures 1 and 2).

Bernard thought that the puncture of this centre at the medulla was responsible of dilatation of the hepatic vessels. The augmentation of the blood flow to the liver would enhance the activity of the glycogenolytic ferment. Bernard also found



Figure 1. *One Lesson. Claude Bernard (1889)* by Leon Lhermitte (1844-1925). In the picture, CB is demonstrating the role of the vasomotor nerves of a rabbit

that cutting the vagi in the neck arrested the sugar release by the liver, arguing that afferent impulses must pass up the nerve to stimulate the nervous center at the medulla.⁶ Macleod investigated the evidence, for or against a nervous control of the glycogenolytic function of the liver, in well-fed dogs anesthetized with ether. He observed that in this circumstance, which precautions taken against the development of dyspnea, the stimulation of the central end of the vagi causes no distinct hyperglycemia. Furthermore, when every precaution was taken against dyspnea, stimulation of the spinal cord at any level failed to produce hyperglycemia, denying the existence of efferent glycogenolytic fibres. On the contrary, the stimulation of the left splanchnic nerves produced marked hyperglycemia and glycosuria, demonstrating that, *when every precaution is taken against asphyxia, glycogenolytic fibers are demonstrable with certainty only in the greater splanchnic nerves; there is no evidence that stimulation of the central*

*end of the vagus can reflexly produce hyperglycemia.*⁷ Therefore, these experiments denied the existence of afferent or efferent nerve fibres connecting with the hypothetical diabetic centre in the medulla oblongata.

Then, Macleod extended the investigation to obtain more complete data regarding the role of the greater splanchnic nerves on glycogen metabolism. He performed experiments in anesthetized dogs with ether, with a tracheal cannula inserted allowing washed oxygen passing down the respiration tube, and arterial cannulae introduced into the carotid and femoral arteries. The greater splanchnic nerve was exposed on the left side and electrodes placed in position on it. Blood sugar samples from the femoral cannula were measured by the method of Waymouth Reid. The experiments investigated the influence of faradic stimulation of the left greater splanchnic nerve on the amount of sugar in blood, the rate of urine excretion, and the amount of

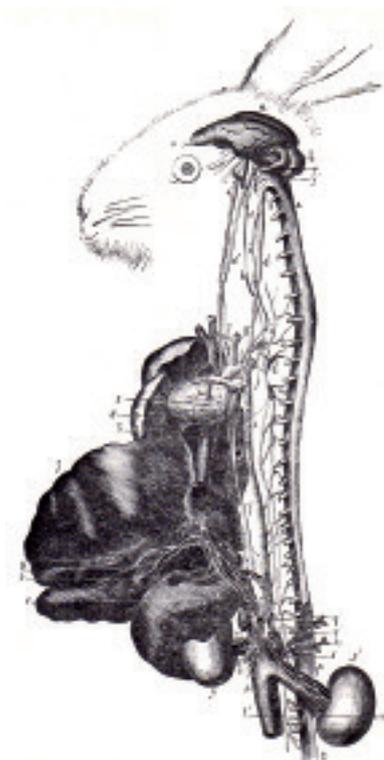


Figure 2. C. Bernard. Cerebrospinal and splanchnic connections to explain (in the rabbit) the mechanisms of artificial diabetes (Adapted by JJ Peumery. *Histoire Illustrée du Diabète*, Éditions Roger Dacosta. Paris, 1987)

sugar in urine. The results corroborated previous observations. Stimulation of the great splanchnic nerve induced more or less marked hyperglycemia, already established within half an hour. When the stimulus was maintained for several hours, the hyperglycemia reached a maximum in about 2 hours, after which it declined.⁸

The following step was to investigate the influence of stimulation of the great splanchnic nerve on glycogenolytic activity of the liver when the experimental animal was deprived of blood supply. The hypothesis to be tested was that the hyperglycemia which follows the stimulation of the great splanchnic nerve might be accounted for by the local anemia in

the liver generated by stimulation of vasoconstrictor fibers. The experimental design investigated the rate of disappearance of glycogen in pieces of liver removed during stimulation of the splanchnic nerve versus in pieces removed without such stimulation. In one series of experiments, the portal vein was anastomosed with the vena cava. In another series, besides making an anastomosis between the vena porta and the vena cava, the hepatic artery was ligated. The results of these experiments allowed to conclude that the absence of the portal blood supply stimulated glycogenolysis, which became much more marked when there were deficient hepatic artery supply. Therefore, stimulated fibres are not secretory in nature, they might be vasoconstrictor fibres.⁹

Macleod also carried out experimental research to assess the non-existing evidence of depressed glycolytic power in eviscerated animals, either normal or with experimental diabetes.¹⁰ Similarly, he conducted investigations on the distribution of glycogen over the liver immediately and some time after death. He found that the distribution of glycogen over the liver was not uniform. It varied by from 5 to 7 per cent, being not greater during absorption than at other times. He observed that artificial stimulation of the splanchnic nerve did not accelerate post-mortem glycogenolysis in intact liver. After death, there is, usually but not always, an acceleration in the rate of glycogenolysis, which varies in different lobes. The time of onset of *post-mortem glycogenolysis* is established within twenty minutes after death. Once established, post-mortem glycogenolysis proceeds at a uniform speed for several hours after death,

being dependent on the amount of remaining glycogen.¹¹

Macleod also performed histological research on liver glycogen: microscopic slides showing sections of the liver from normal rabbits and from rabbits with experimental diabetes. In normal animals, glycogen (stained by the modified Best's carmine method) was present only in the liver cells and never in the sinusoids or sublobular veins. On the contrary, in the liver of diabetic animals red-staining glycogen masses were present in the sublobular veins.¹² Macleod took benefit of a several weeks' residence at the Marine Biological Station (East coast of Vancouver Island, at Nanaimo, B.C.) to investigate the amount of glycogen (Pflüger method) in selected varieties of marine animals. He could observe that in all cases, the glycogen content of heart muscle was several times greater than that of other muscles, and some times even greater than that of the liver.¹³

After all of these research activities, Macleod agreed upon the existence of a glycogenolytic enzyme, glycogenase, produced by the liver, and that the release of glucose by the liver, either ante-mortem or post-mortem is due, not to variable amounts of glycogenase, but to variations in the activity of inhibitory mechanisms which holds it in control. The nervous control of the glycogenic function is activated by the reflex act having its center located at the fourth ventricle. The efferent part of from this center to the liver is by way of the great splanchnic nerves. The nerve fibers may have no direct influence on the amount of glycogenolytic enzymatic activity, but may exercise a control on those conditions

which retard or inhibit its activity. The glycogenolytic function of the liver is affected by considerable changes in its portal blood supply. Nevertheless, it appears highly improbable that the changes in blood supply produced by stimulation of the splanchnic are of sufficient magnitude to excite glycogenolysis. Therefore, the driven conclusion is that the nervous influence must be either on the production of the enzyme or in the inhibiting mechanism. Finally, for Macleod the glycogenic function of the liver was at least as important as the glycolytic action in the tissues, recommended it should be more carefully investigated as to its participation in the cause of diabetes.

Significance of Lactic Acid in intermediary metabolism

In a group of publications, Macleod showed his interest in investigating the sources of lactic acid in blood when there is a limited supply of oxygen. In particular, he studied the production of lactic acid following the occlusion of the blood supply of the liver. He reached the conclusion that lactic acid is readily produced from glycogen in the liver as a result of local stagnation of blood flow.¹⁴ Other investigations were focused on the analysis of lactic acid production following the intravenous administration of alkaline solutions or glucose. For this purpose, Macleod carried out various experiments measuring lactic acid levels in blood drawn from the pancreatico-duodenal vein, before, during and shortly after the injection of an alkaline solution of dextrose, and also after a subsequent injection of a similar amount of dextrose in faintly acid solution. The results indicated that the factor upon which

the lactic acid production depends was the alkalinity of the solution rather than the actual amount of dextrose injected. The injection of a faintly acid solution of dextrose did not cause any perceptible change in the lactic acid content of the blood. Whereas the dextrose injected in acid solution caused no increase in lactic acid levels, a similar dextrose injection in alkali solution caused a marked increase.¹⁵

Effects of the administration of glucose on the sugar-retaining ability of liver and muscles

Macleod was moved by various observations of pioneers like Murlin and Kramer, Kleiner and others, regarding the observed effects during/ after the intravenous administration of glucose on glycosuria, disappearance of glucose from blood, and the hypoglycaemic influence of alkaline solutions. He decided to investigate the effects of a constant rate of intravenous glucose administration. In most experiments the amount of glucose employed for injection were below the normal tolerance limit without causing glycosuria (usually 0.9 g of glucose per kilogram of weight and per hour). The results of these experiments allowed to conclude all the following:

- When dextrose is injected in the portal vein into anesthetised dogs, occurs a rapid increase in blood glucose levels. The rise reaches a certain concentration, after which the blood sugar level remains constant.
- The behavior of the blood glucose profile from the portal vein, vena cava and iliac veins is very similar. The sugar-retaining powers of the liver and muscles are not sufficiently developed to cause percep-

tible changes in the inflowing-outflowing blood of these tissues. The sugar-retaining power of the liver is approximately equal to the muscles of the hind limb.

- The administration of large amounts of glucose is followed by an increase of H-ion concentration of the blood. When an alkaline solution (sodium carbonate) is intravenously administered in sufficient amount to lower the H-ion concentration of the blood, there is a distinct decrease in blood glucose, with similar decline in the portal vein, vena cava and iliac veins.¹⁶

The relationship between nervous and hormone control of the respiratory center

Interesting experimental observations were carried out by Macleod and Page regarding the activity of the respiratory center in cats after decerebration (Sherrington's method). In this animal model, the section of one vagus did not have any effect on breathing but the section of both sides caused a decline of the respiratory rhythm along with increased the depth of respiratory motion. Increasing the percentage of carbon dioxide in the inspired air had also the same stimulating effect on respiration before and after section of vagi, although with high percentages of carbon dioxide, the respirations after section of the vagi become slower and the minute volume ceases to increase and may decline. These findings might be considered of interest in interpreting the inconsistent information registered in the activity of respiratory centers in laboratory animals by various forms of sensory stimulation and by alterations in the composition of the blood, due to the fact that these ob-

servations are usually obtained on animals under varying degrees of anesthesia.¹⁷

Statements of J.J.R. Macleod regarding the history of the development of pancreatic extracts in the Department of Physiology, University of Toronto (1921-1922)

All the following paragraphs have been selectively extracted from written documents by J.J.R. Macleod, published in the Bulletin of History of Medicine.¹⁸

Researches Leading to the Discovery of Insulin

Some time early in 1921 F.G. Banting called on me in my room in the Physiological Department of the University of Toronto and said that he had been giving part of his time to assisting in teaching and research in the physiological department of Western University at London, Ontario. In the course of his reading he had been struck with the indirect nature of the evidence upon which rests the theory that the Islets of Langerhans of the pancreas furnish an internal secretion the presence of which is in some way related to the occurrence of diabetes. He told me that he discussed this with Professors Miller and Crane in London and that they suggested his consulting me.

As everyone familiar with the problem has for long realized, the preparation of an extract of pancreas containing the active principle (or internal secretion) that control the metabolism of sugar is complicated by the presence in such extracts of digestive ferments which could destroy the active principle. This point immediately came up in our discussion and Dr. Banting made the sug-

gestion that it might be possible to circumvent these ferments by using duct-ligated pancreas. As stated in the first paper published on the work he formed this idea while reading in a textbook of surgery. I also told Dr. Banting that it would be useless to attempt this work unless he was prepared to give up all his time for several months to the problem, but that if he agreed to do this, I would place every facility at his disposal and show him how the investigation should be planned and conducted...

...He talked the matter over with Dr. Clarence Starr who a month or so after Banting's visit called me to ask whether I thought the problem was sufficiently hopeful of a favourable outcome to warrant Banting taking the step I had suggested. I told Dr. Starr that although it was taking considerable chances I thought the research well worth proceeding with. Some time later Banting wrote me that he had almost decided to come to Toronto and I replied that I would help him all I could to proceed with the investigation.

He arrived about the middle of May 1921. I found that Dr. Banting had only a superficial text-book knowledge of the work that had been done on the effects of pancreatic extracts in diabetes and that he had very little practical familiarity with the methods by which such a problem could be investigated in the laboratory...

...I worked out with Dr. Banting a plan of investigation the first step of which was to render one or two dogs diabetic by extirpation of the pancreas so that he might make himself familiar with the cause of this condition in animals untreated with extract. At the same time I advised

him to tie the ducts in several other animals so that the gland might be suitably degenerated when the time came to use extracts. I advised him to use the Hedon method for extirpation of the pancreas, gave him the necessary references, and assisted him in the first operation. Since he had no practical knowledge of how to conduct the estimations of sugar in blood and of nitrogen and sugar in urine, upon the accuracy of which the whole investigation depended, I arranged for one of my research fellows, C.H. Best, to collaborate with him.

I left for Britain in the middle of June...

...On my return to Toronto at the end of September I found that Banting and Best were dissatisfied with the facilities at their disposal, and early in October they formally demanded of me that I improve them. Dr. Banting said that if I did not do so he would apply to the Rockefeller Institute or the Mayo Clinic for facilities. I agreed to give Dr. Banting the half-time of one of the laboratory boys, Arthur Lamb, a separate room and every other facility I could. Meanwhile, Professor V.E. Henderson had generously arranged to offer Dr. Banting a temporary position as teaching assistant in his department with a salary...

...About the middle of November I asked Banting and Best to give their results at the Journal Club. Banting requested me to introduce the subject, which I did, giving briefly some of the historical relationships of their work. I was told, in January (1922), that Banting considered that in doing this I did not sufficiently indicate the share he had in the work. Had I been

told of this attitude of Banting at that time, it would have served to warn me of his peculiar temperament and of his entirely unwarranted suspicions that I was trying to receive the credit for the results he had obtained...

... A week or so later I suggested that the work was now suitably advanced to warrant publication, and I showed Banting and Best how the results should be compiled. When their draft was ready I spent considerable time going over it and made several alterations in the manner of presentation. When finally the manuscript was ready Banting asked me if I wished my name to appear along with his and Best's, and my reply was that I thanked them but could not do so since it was their work and "I did not wish to fly under borrowed colours"...

...It was at this stage that Dr. Banting invited Dr. J.B. Collip to collaborate in the investigation, especially in connection with the preparation of pure extracts. Dr. Collip immediately started this work extracting adult ox pancreas by means of alcohol...

...At the meeting in new Haven Banting presented the paper at the last conjoint session. Banting was very nervous, and it was evident that he had not succeeded in convincing all of his audience that the results obtained proved the presence of an internal secretion of the pancreas... Although chairman of the meeting, I took part, laying stress on the frequency of direct relationship between the injections and the lowering of the blood sugar and on the prolongation of life of two treated animals...Dr. Graham told me later

that Banting considered that I had deliberately so discussed his paper at New Haven as to convey the impression that I was entirely responsible for the origination of this piece of work and that he and Best had merely acted as research assistants. If this was so, it was entirely unintentional on my part, my object being to persuade the audience of the real value of the investigations...

...At this time (January) Collip reported to me at lunch time practically every day the progress he was making in the preparation of the extract, and at Banting's repeated solicitations I persuaded Dr. Duncan Graham to give him the opportunity in his clinic to try the extract on a case of diabetes. Some extract prepared by Banting and Best about this time or previously was injected subcutaneously into a boy, under the immediate charge of Dr. W.R. Campbell, with the result that the blood sugar and urinary sugar, I think, were temporarily reduced, but several abscesses developed at the places of inoculation. This, of course, made it impossible to continue the use of such extracts, and it served to spur on Collip to secure purer extract, which he succeeded in doing by the method now in use. Collip is entirely responsible for his work, and it is unfair and unjust for Banting and Best to rob him of any of the credit by saying that they told him of the percentages of alcohol at which the active principle was soluble. Collip denies that they gave him any information that was of use in this connection and they never communicated any such to me...

...As a result of Collip's researches a non-irritating highly potent prepara-

tion of insulin was supplied to the Medical Clinic and was used in the cases reported in the Canadian Medical Journal in March.

I wish to emphasise here that the development of the investigation at this critical stage depended on the collaborative effort of several workers:

- 1. To make an extract*
- 2. To make it safe for clinical use*
- 3. To find a practical method for testing the potency of the extract*
- 4. To demonstrate that the action of the active principle in the extracts on animals was more far-reaching than lower the blood sugar and the urinary sugar in depancreated animals*

J.B. Collip (1892-1965) Biographical Sketch

O.H. Warwick, Vice-president of Health Sciences, and Dean, Faculty of Medicine, University of Western Ontario, London, Ontario, Canada, wrote the obituary of James Bertram Collip, published in the Canadian Medical Association Journal.¹⁹ Bert Collip was born in Belleville, Ontario, on November 20, 1892. He entered Trinity College in Toronto, at the age of 15 years. He received a PhD in Biochemistry at the University of Toronto in 1916, being Prof. A.B. Macallum his main supervisor. He married to Ray, in 1915, and he became a Lecturer in Biochemistry at the University of Alberta, Edmonton. In April 1921, he joined the University of Toronto with a Rockefeller Foundation Travelling Research Fellowship, to work under Professor JJR Macleod. In the summer of that year, he carried out special studies on anaerobic respiration in molluskus at the Marine Biological Laboratory at

Wood's Hole on Cape Cod. This experience allowed him to use the recently published micromethod of Shaffer-Hartman for determination of blood sugar.²⁰ In mid-December 1921, he started to work in Toronto in the process to purify extracts from whole beef pancreas. He was able to estimate the potency of pancreatic extracts by inducing hypoglycaemia in rabbits, and investigated the effects of insulin on ketone bodies and hepatic glycogen. Furthermore, he was the first investigator who succeeded in generating a pancreatic extract pure enough for administration to diabetic subjects.

On January 23, 1922 the injection of insulin to Leonard Thompson was both effective and well tolerated. Collip remained as Assistant Professor of Pathological Chemistry at the University of Toronto, until returning to Edmonton as Head of the Department of Biochemistry, University of Alberta, in May 1922. In 1926 he obtained the medical degree. In 1927 isolated parathyroid hormone which made a substantial progress in the knowledge of the physiology of parathyroid glands. He was the successor of Professor A.B. Macallum as Chair of the Department of Biochemistry, at McGill University, in Montreal, Ontario. In Montreal he was author of many articles, mainly related to endocrine physiology of pituitary gland, ovary and placenta. After Banting's death, Collip was nominated Chair of the Canadian Research Council's Associate Committee on Medical Research, position he maintained for 16 years. In 1947 became Dean of the Faculty of Medicine, Professor and Head of the Department of Medical Research at the University of Western Ontario, joining Dr. R.J. Noble in his faculty staff.

Collip received many honorary degrees (Harvard, London, and Oxford Universities, among others). Those that knew him well admired his humility and kindness; publicity of any kind embarrassed him. He died on June 19, 1955. He was the father of three children: two females and one male.

Summary of Collip's research activities prior to the first clinical use of insulin

Based upon previous observations about the presence of "peristaltic hormones" in various tissues, Collip performed extensive investigation on the effects of extracts from many tissues on the stimulation of smooth muscle preparations, mainly isolated mammalian intestine and uterus, as well as heart muscle. For this purpose the movements of the contractile response of the muscle strip were recorded. Main observations depicted a definite stimulatory effect of various tissues upon isolated intestinal and uterine musculature. Extracts of heart, spleen, pancreas, testes, anterior and posterior lobe of the pituitary body, thymus, thyroid and parathyroids antagonized the inhibitory action of adrenalin on these organs. The primary effect of pancreatic extracts was depression of the duodenal musculature.²¹

In additional experiments, Collip investigated the vasoactive effect in vivo of various tissue extracts (pancreas, thymus, corpora lutea, anterior pituitary, testes, parathyroid glands), and its antagonism to the vasodilator effect of adrenalin. Experimental animals used for these experiments were dogs and rabbits, investigated under ether anesthesia and a cannula inserted into the left carotid artery. Main results showed the following: a) The fall in blood pressure induced

by small doses of adrenalin was antagonized by tissue extracts; b) The rise in blood pressure induced by increased doses of adrenalin was augmented and prolonged by the administration of tissue extracts; c) The observed antagonism of the depressor action of adrenalin by tissue extracts was of short duration²².

As Cannon and Lyman had demonstrated that under different circumstances small doses of adrenalin might have opposed effects on the blood pressure, and in previous work Collip had confirmed the antagonism of tissue extracts on the vasodilator effect of adrenalin, Collip investigated in another series of experiments the reversal of depressor actions of small doses of adrenalin in anesthetized dogs. In the experimental design, a tracheal cannula was inserted to allow the administration of the anesthetic, and also cannulas were inserted in both the left carotid artery and the left external jugular vein. Blood pressure was recorded with the use of a mercury manometer. Injections were made through the jugular cannula and both vagi were cut. It was noted that reversal of the depressor action of small intravenous doses of adrenalin was achieved after increasing the anesthetic. This effect was reversed after resuming light anesthesia. If the animal was not under the influence of ether, the effect of a small dose of adrenalin was a fall in blood pressure. The same dose of adrenalin given after full ether was associated to a rise in blood pressure. Collip also found that reversal of the depressor action of a small dose of adrenalin could be observed by sudden increase in the alkalinity of the blood by administering a large dose of sodium carbonate. The opposite effect was registered following the

intravenous administration of acid sodium phosphate. In addition, the pressor response to a larger dose of adrenalin was augmented by an increase in the alkalinity of the blood and was decreased by the decrease in the alkalinity. These results suggested that hydrogen ion concentration determines the vascular reactivity, whereas the drug acts upon the myoneural junctions of the sympathetic nerve fibers²³⁻²⁴.

The main contribution of JB Collip to the treatment of diabetes: The purification of the pancreatic extract

The research activities leading to the development of insulin by the researchers of the Department of Physiology, University of Toronto, directed by Prof. J.J.R. Macleod, followed four consecutive phases:

1. The demonstration that the pancreas secreted an active principle responsible of reducing hyperglycemia and glycosuria in depancreatised animals
2. The isolation of a pancreatic extract with sufficient purity to allow the continuous administration to human subjects by subcutaneous injection
3. The demonstration that this purified product makes disappear all cardinal symptoms of diabetes when adequate doses are administered
4. The large scale production of the purified extract.

Frederick G. Banting and Charles H. Best, in Professor J.J.R. Macleod's department and laboratory, and under his direction, were already working with the objective to obtain a pancreatic extract, influenced by the idea that the internal secretion of the

pancreas might be destroyed by proteolytic enzymes during the extraction procedure. To avoid this event, they tried to prepare watery extracts of degenerated glands weeks after the ligation of the pancreatic duct. Collip became associated to the team in December 1921, with the focus of developing a method with enough purity and stability of the extract to allow the continued use in diabetic patients. At that time, extracts were prepared by macerating the gland with an equal volume of alcohol, filtering, adding one drop of glacial acetic to each 100 ml of the filtrate, followed by concentrating the filtrate to about one-fifth in a vacuum still. The active principle was contained in the protein fat residue of the straw-colored fluid removed by filtration.

Banting and Best prepared a sterile extract, using this technique, and delivered it to Drs. Campbell and Fletcher of the Medical Clinic, Toronto General Hospital. They tested out this extract on a few diabetic patients. In one case, they could observe a 25 per cent decrease in blood sugar levels, after its administration. Nevertheless, the patient developed aseptic abscesses at the sites of injection, due to the high protein concentration. At that point, the extract was useless for continued administration to patients.

Then, the researchers of Toronto investigated the effects of powerful extracts on normal animals, observing a definite fall in blood glucose levels in rabbits. Some of them developed convulsive seizures, coma and death. Complete recovery was quickly achieved if glucose was provided. Hypoglycemia could not be evident in dogs under the effect of ether anesthesia.

Table 1. J.B. Collip: The original method as used for the isolation of insulin in semipure form for the treatment of the first clinical cases (J Biochem. 1923;55:40-1)

- The method applied in the preparation of the first insulin used in the treatment of clinical cases was developed by the writer during December and January last. In the critical first weeks of clinical trial of insulin the preparation of the extract was carried out exclusively by the writer...
- The original method was as follows:
 - To a small volume of 95 per cent ethyl alcohol, freshly minced pancreas was added in equal amount. The mixture was allowed to stand for a few hours with occasional shaking. It was then strained through cheese-cloth and the liquid portion at once filtered
 - Some hours after this final precipitation the precipitate was caught on a Buchner funnel, dissolved in distilled water, and then concentrated to the desired degree by use of the vacuum still. It was then passed through a Berkefeld filter, sterility tests were made, and the final product was delivered to the clinic
 - The essential points relating to the extract prepared as outlined above are:
 - (1) It contains only a minimum of protein
 - (2) It is practically salt-free and can readily be made isotonic
 - (3) It is lipoid-free
 - (4) It is almost free from alcohol-soluble constituents
 - (5) It can be administered subcutaneously without fear of any local reaction

The next step was to make progress in the purification of the extract for the treatment of clinical cases. Collip was able to achieve this goal after intensive work during December 1921 and January 1922 (table 1).²⁵ This method was developed by Collip while he still belonged to the Department of Pathological Chemistry, University of Toronto. During the first few weeks of clinical trial, the preparation of the extract was carried out exclusively by him

Statements of J.B. Collip regarding the history of the development of pancreatic extracts, before being used in clinical trials

The following paragraphs contain some selected extracts from the own written document of James B Collip regarding the main steps in the discovery of insulin, published in August 1923,²⁶ with the intention to complete the information on this matter prior to the implementation of clinical trials with insulin (which

will be the main objective of the next article of this compiled series).

...Hedon showed that, if a portion of the pancreas was first grafted successfully under the skin of the abdomen, the rest of the gland could subsequently be removed without the resultant manifestation of diabetes. Severe and quickly fatal diabetes was produced, however, if the grafted portion of the gland was removed at a later operation. Numerous attempts were later made by various investigators to obtain some direct evidence of the presence of the internal secretion or hormone in the pancreas or in the blood emerging from the gland. The most noteworthy investigation of this nature was carried out by Zuelzer. Murlin and Kramer, Kleiner, and Paulesco observed a transitory reduction in the percentage of blood sugar and in the sugar excretion of depancreatized dogs, following the intravenous injection of aqueous extracts of the normal pancreas...

...Very definite proof that the pancreas does produce an internal secretion was furnished by Forschbach. This investigator found that the extirpation of one parabiotic dog was followed by slight glycosuria only...the internal secretion of the remaining pancreas of the other (normal) parabiotic dog sufficed for the needs, not only of itself but also of its depancreatized parabiotic mate. After separation the animal which had been depancreatized developed diabetes...

...The next forward step in the research was the isolation of the hormone by the writer in a sufficient pure and stable form to allow of continued use by subcutaneous injection in the human subject. This was followed at once by the clinical trial of the purified hormone...The results obtained on depancreatized animals, lowering blood sugar, decrease in sugar excretion, disappearance of ketones and rise in respiratory quotient were duplicated in this investigation on the diabetic human subject and insulin therapy had become an established procedure...

For the development of a successful large scale process of manufacture the Eli Lilly Co. of Indianapolis, who later cooperated with the Toronto group, are largely responsible...

...A very definite step forward in the further purification and stabilization of insulin has been made by Doisy, Somogyi and Schaffer, and by Dudley, Doisy, Somogyi and Schaffer finding that insulin may be precipitated from solution by adjustment of the reaction to the isoelectric point...

...Some of the chief contributions of the Toronto group have already been indicated. Up to the time that definite

clinical investigation was begun the following salient points have been established:

1. Insulin causes a lowering of the blood sugar of both normal and diabetic animals.
2. Insulin produces convulsions in normal animals as a result of low blood sugar (0.045 per cent critical level.)
3. Hypoglycemic symptoms can be definitely controlled by administration of glucose and to a degree by adrenalin.
4. The liver of a depancreatized animal can store glycogen under the influence of insulin.
5. The respiratory quotient of a depancreatized animal is raised by insulin, thus indicating utilization of carbohydrate.
6. Ketosis in depancreatized animals is abolished by insulin.
7. Depancreatized animals injected with insulin can retain a considerable amount of administered glucose

...There was as yet no absolute proof that the hormone of the pancreas was elaborated by the islet cells. Macleod, taking advantage of the discovery of Rennie²⁷ that the islet tissue of Teleostian fishes is collected into nodules which are often encapsulated and thereby separated from the zymogenous tissues, secured practically pure islet tissue from the angler (*Lophius*) and the sculpin (*Myoxocephalus*). He was able to prepare very potent extracts of the zymogenous tissue of the same fishes to be quite inert²⁸ (figure 3).

Macleod has, therefore, definitely established that islet tissue is the source of insulin... (underline by the authors) (figure 4)

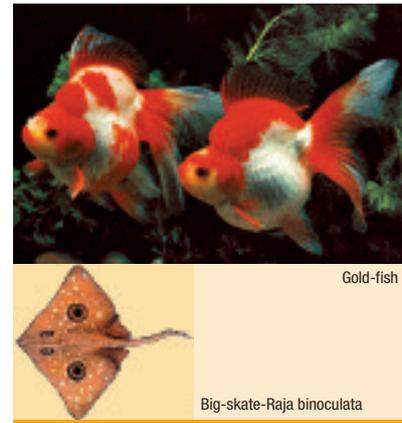


Figure 3. Teleostian fishes



Figure 4. Macleod J.J.R. The Source of insulin. *J Metab Res* 1922;2:149-72

Macleod and Collip's contributions to the discovery of insulin: the appraisal of two expert historians

Banting, Best and Macleod labored under the false idea that the pancreatic extracts contained powerful proteolytic enzymes which could digest or destroy any internal secretion also present. They ignored that Heidenhain as early as 1875 had shown that extract of fresh pancreas have no

Table 2. J.H. Pratt: A reappraisal of researches leading to the discovery of insulin (J History Med. 1954;9:281-9)

	Depancreatized dog		
	Blood sugar (Mg.%)	Fall in blood sugar (Mg.%)	Intravenous injection
August 15			
Noon	300	70	10 cc extract "degenerated" pancreas
1 p.m.	230		
August 16			
10 a.m.	300		10 cc "degenerated" pancreas extract + 0.1% HCl
11 a.m.	180	120	
August 17			
6 p.m.	300		10 cc normal gland extract
7 p.m.	170	130	
August 18			
Midnight	220		10 cc normal gland extract + 0.1 HCl
4 a.m.	150	70	

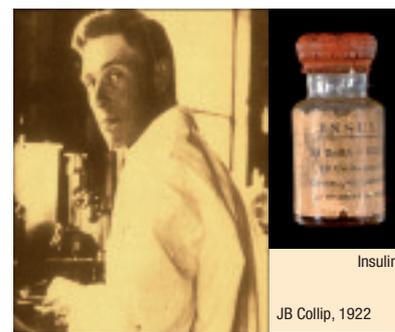
proteolytic activity but a zymogen which under certain conditions form an active ferment.²⁹ Bayliss and Starling stated that Langley showed that the proteolytic ferment was in the fresh pancreatic gland in the form of the precursor trypsinogen.³⁰ Banting originated the hypothesis that the failure to isolate the internal secretion of the pancreas had been due to the destruction by the ferments liberated during the process of extraction. For this reason, he devised an experimental procedure to avoid that destruction, consisting in tying the pancreatic duct, as advised by Barron.³¹ Apparently, Banting died without ever having learned that in his experiments he had attempted to destroy something that did not exist, an active proteolytic enzyme in the fresh pancreas. E.L. Scott held the same view when he started similar experiments already in 1912 in Chicago,³² although later on he abandoned the ligation of the ducts, considering the method futile and impractical. John Murlin, University of Rochester, also believed that the action of ferments should be eliminated. For this con-

cern, he extracted the pancreas with strong HCl, to destroy trypsin.³³

On the contrary, various research groups developed active extracts directly from fresh animal pancreas. In their multiple experiments, Banting and Best really worked with essentially normal glands. From their own work, J. Pratt elaborated the following table (table 2).

The information presented in the table clearly illustrates that the extract of the normal pancreas was equally active in decreasing blood sugar as the so called "degenerated gland". When Banting presented at the meeting in New Haven the result of the work carried out in association with Best for eight months, the two Canadian investigators had not provided any more advanced step than G Zuelzer fifteen year before.

In December, 1921, Macleod brought JB Collip to the team. His knowledge and skills as outstanding biochemist allowed in a few weeks to

**Figure 5.** Purification of the pancreatic extract

achieved the key progress: the precipitation of insulin by 95% or absolute ethanol rendered the extract non-toxic; the powerful extract could be, from now on, to be successfully applied to the treatment of human diabetes (figure 5).³⁴

The most relevant historian expert on the Discovery of Insulin, the University Professor Michael Bliss (University of Toronto), coincided with the version of J Pratt, regarding these episodes.³⁵

...It was soon realized that Banting's duct ligation procedure was not necessary for the preparation of effective extract. It could be made just as effectively from chilled, fresh beef or pork pancreas...the problem was that no kind of extract worked consistently. Banting and Best's notebooks reveal that their experiments were often frustratingly ineffective. However, by that time a fourth researcher had been added to the team. James B. Collip was a skilled biochemist, a professor of the University of Alberta who had taken research leave and had been working in Toronto with Macleod on a quite different problem. Collip was added to the team at Banting's request in December 1921. He began making his own extract, drawing on Banting and Best's meth-

ods but adding his own, and had much better success.

...On January 11, 1922, Leonard Thompson, a charity patient who was 14 years old and reduced to 29 kilos from his diabetes, was given pancreatic extract made by Banting and Best. The extract failed to relieve significantly the cardinal symptoms of the boy's diabetes and its administration was immediately discontinued. Twelve days later, however, on January 23, Thompson was given extract made by Collip-who had developed a process that enabled him to remove many of the contaminants in Banting and Best's extract- and it worked remarkably well to reduce urinary and blood sugar, remove ketones, and generally treat the boy's diabetes. ■

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