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Discovery of Insulin is a Great Achievement for the Diabetes Patients

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Abstract

Before the discovery of insulin, a diabetes patient must face an unavoidable death sentence. The discovery of insulin is a milestone in medical science, and it is really a revolutionary work for the welfare of the diabetes patients. In parallel it creates controversies and disputes among scholars; and disappointments, failures, and hopes in the way of discovery. The leading work to the discovery of insulin has been started in 1921 by the Canadian medical scientist and physician Frederick Grant Banting, who had no research experience, no publications, and not even a doctorate degree. He has started research through the supervision of Professor John James Richard Macleod; with two lab assistants of the bachelor degree students named Charles Herbert Best, and Edward Clark Noble, and ten dogs as experimental devices. The first applications of insulin in human body became possible in 11 January 1922 on Leonard Thompson, a 14-year-old boy who was suffering from diabetes mellitus. This study tries to discuss the aspects of discovery of insulin and the further continuous development of it for the welfare of the diabetes patients.

Keywords: insulin, β-cells, Banting, Best, Collip, Macleod, Paulescu

1. Introduction

When we eat, our blood glucose level rise to high level, and if it crosses the normal range we need to reduce for maintaining a lengthy and healthy life. The insulin hormone is 51-residue anabolic protein, which is synthesized by β-cells in the Islets of Langerhans of the pancreas, and can reduce the glucose efficiently (Bliss, 1982). Insulin is secreted when the level of blood glucose rises. The excess glucose is stored in our body either as glycogen (liver) or as triglycerides (adipose). If high blood sugar cannot be controlled for a long-time, diabetes mellitus (DM) is developed (Mohajan & Mohajan, 2023a, b). DM is a metabolic disorder characterized by hyperglycemia, hyperlipidemia and hyper-aminoacidemia that is either for inadequate insulin production, or for the body cells do not respond properly to insulin, or both (García, 2017; Mohajan & Mohajan, 2023d, e). If the disease DM is left undiagnosed or poorly controlled for the long-time, it may damage vascular and nervous system leading to devastating diabetes complications, such as retinopathy, neuropathy, nephropathy, amputation and cardiovascular diseases. As a result, many complicated problems may be happen in vital organs, such as in heart, vasculature, eyes, kidneys, and nerves (WHO, 2002; Mohajan & Mohajan, 2023c). International Diabetes Federation (IDF) shows that in 2019, diabetes caused 4.2 million deaths, and 463 million adults aged between 20 and 79 years old are living with diabetes that costs $720 billion. It is expected that diabetes patients may rise up to 700 million by the 2045. If the diabetes infected patients left untreated can lead to life-threatening complications, and consequently early death may increase (Galicia-Garcia et al., 2020).

Insulin is a peptide hormone that is produced and released by β-cells of the pancreatic islets from the INS gene, located on chromosome 11 (Tokarz et al., 2018). It plays a central role in the regulation of human metabolism. It finely tunes the metabolism of carbohydrates, fats, and protein inducing the uptake of glucose from the blood

into fat, liver, and skeletal muscle cells, and also reduces blood sugar level (Vecchio et al., 2018). Production of glucose and secretion by the liver is strongly inhibited by high concentrations of insulin in the blood (Sonksen & Sonksen, 2000). The human insulin protein is composed of 51 amino acids, and has a molecular mass of 5808 Da. It is a heterodimer of an A-chain and a B-chain, which are linked together by disulfide bonds (Tof, 1994).

Discovery of insulin is one of the most important achievements and scientific milestones in medical science that ensures the survival of millions of people with ever improving treatments of the diabetes mellitus patients and also provides a new quality of life (Bliss, 1982). At present there are some peptide hormones to treat the patients, such as recombinant insulin, insulin analogues, oral insulin, and inhaler insulin (Anwer et al., 2015).

2. Literature Review

The literature review section is an important and an introductory portion of a research, where works of previous researchers are highlighted (Polit & Hungler, 2013). It enhances the activities of researchers through the understanding the core idea of the subject area that has been carried out before (Creswell, 2007). It is a secondary source and does not report a new or an original experimental work (Gibbs, 2008). A good literature review can ensure that a proper research question has been asked and a proper research methodology has been chosen (Baglione, 2012; Torraco, 2016).

Celeste C. Quianzon and Issam Cheikh review the history of the discovery and further development of insulin. They have demanded that before the availability of insulin, the life expectancy of children with diabetes mellitus was short and the prognosis for the adult onset diabetes was very poor (Quianzon & Cheikh, 2012). Dimitrios T. Karamitsos has mentioned that many researchers had tried to isolate insulin from animal pancreas, but Frederick Banting, a young surgeon, and Charles Best, a medical student, were the ones that succeeded (Karamitsos, 2011). Peter Diema and his coauthors have expressed that the discovery of insulin in Toronto in 1921 came from Frederick G. Banting, who worked under the direction of John J. R. Macleod in the Institute of Physiology at the University of Toronto. He was assisted in his experimental program by the student Charles H. Best (Diema et al., 2021).

Sisir K. Majumdar gives case details of the first patient, a 14-year-old boy named Leonard Thomson, who is severely infected with diabetes, and who had reached at the end stage of life; Frederick G. Banting and Charles Best have first used insulin upon him in 11 January 1922 (Majumdar, 2001). Hertzel C. Gerstein and Christopher J. Rutty described that the miracle of insulin therapy for type 1 diabetes is benefited millions of people worldwide on a daily basis. Before the discovery of insulin, children and teenagers diabetes patients are wasted away and die within two years. But at present people can manage this disease and live with through a long and productive life (Gerstein & Rutty, 2021).

Klaus Gast and his coauthors have provided the comparison of the dissociation kinetics of rapid acting insulin lispro, aspart, glulisine and human insulin under physiologically relevant conditions that will be helpful for optimizing formulation conditions of rapid-acting insulin. They have obtained glulisine forms compact hexamers in formulation even in the absence of Zn^{2+} (Gast et al., 2017). Irl B. Hirsch and his coauthors have investigated how the biochemical properties of endogenous insulin were exploited to either shorten or extend the time-action profiles of injectable insulin by varying the pharmacokinetics and pharmacodynamics. They have designed various insulin and formulations to solve the challenges of insulin replacement for the treatment of patients (Hirsch et al., 2020). Ignazio Vecchio and his coworkers have provided a comprehensive overview of main steps of finishing into insulin discovery, including recent advancements, such as personalized and individualized insulin therapy (Vecchio et al., 2018).

3. Research Methodology of the Study

Research is a vital and significant device to the academicians for the leading in academic world (Pandey & Pandey, 2015). A well-developed outline of the study and an efficient understanding are essential to reach the goal of a research (Tie et al., 2019). Methodology is a guideline to perform a good research that follows scientific methods efficiently (Kothari, 2008). It relates to nature and power to science, truth, and epistemology (Ramazanoglu & Holland, 2002). It is a theory that analyses how research can be done and should proceed efficiently (Harding, 1987). It provides the research design and analysis procedures to perform a good research (Hallberg, 2006). To rationalize the selection of a research methodology, a researcher must understand its philosophical origins and unique characteristics (Rieger, 2019). Therefore, research methodology is the collection of a set of principles for organizing, planning, designing and conducting a good research (Legesse, 2014).

When a researcher goes through a research s/he must go about being as reliable as possible. S/he should apply and demonstrate rigorous collection and analysis methods and systems. On the other hand, validity provides a true measurement, description, and explanation of what it is claiming to be measured (Campos, et al., 2017). Throughout the study we have tried to maintain the reliability and validity as far as possible. We have started our

research with the basic concept of insulin and then we have briefly discussed the structure of insulin, and finally we have discussed the further development of insulin. In this study, we have used secondary data sources that are related to discovery of insulin and development of insulin. We have consulted and analyzed renowned journal articles, printed and e-books of eminent authors, handbooks, conference papers, internet websites, etc. to successfully complete the study

4. Objective of the Study

The main objective of this study is to discuss the aspects of insulin. Some other trivial objectives of the study are as follows:

- to provide the structure of insulin,
- to discuss the discovery of insulin in briefly, and
- to highlight the improvement of insulin.

5. Basic Idea of Insulin

Insulin, a biological product, is a peptide hormone that is produced and released by *β*-cells of the pancreatic islets, which finely tunes the metabolism of glucose, carbohydrates, fats, and protein. It regulates the storage and use of sugar (glucose) by cells in the body (Vecchio et al., 2018). It primarily acts to stimulate glucose uptake by three tissues: adipose (fat), muscle, and liver, which are important in the metabolism and storage of nutrients. In adipose tissue, insulin stimulates glucose and increased esterification of fatty acids with glycerol to form triglycerides (Utiger, 2023).

The term “insulin” was derived from Latin word “insula for islet or island”, and was coined by English physiologist Edward Albert Sharpey-Schafer (1850-1935) in 1916 for a hypothetical molecule produced by pancreatic islets of Langerhans, which looked like islands that controls glucose metabolism. The name “insulin” was proposed in 1909 by the *Belgian* physician *Jean de Mayer* (*1878-1934*) for the unknown substance in the pancreas (Vecchio et al., 2018).

6. Structure of Insulin

Insulin is a small globular protein, and its gene encodes about 51 amino acids; and contains two polypeptide chains: A-chain, composed of 21 residues, and forms a signal peptide; and B-chain, composed of 30 residues and folds into proinsulin (Mukherjee et al., 2018). Two disulfide bridges: Cys^{A7} to Cys^{B7} and Cys^{A20} to Cys^{B19} covalently link chains A and B. In addition A-chain contains an intra-chain disulfide bridge Cys^{A6} to Cys^{A11} (Weiss, 2009). Insulin is developed from a 74 amino acid prohormone molecule called proinsulin, which is comparatively inactive, and under normal conditions only a small amount of it is secreted (Strazza et al., 1985; Gast et al., 2017). The C-chain, which connects A-chain and B-chain, is liberated along with insulin after breakdown of proinsulin precursor (Bell et al., 1980).

The molecular formula of human insulin is $C_{257}H_{383}N_{65}O_{77}S_6$. The A-chain shows two *α*-helical regions at bridge Cys^{A1} to Cys^{A8} and bridge Cys^{A12} to Cys^{A19} which are antiparallel. The B-chain has a central α-helix that covers residues Cys^{B9} to Cys^{B19}, bordered by the disulfide bond on both sides and two *β*-sheets cover Cys^{B7} to Cys^{B10} and Cys^{B20} to Cys^{B23} (Fu et al., 2013).

Proinsulin, insulin, and C-peptide are stored in granules in the *β*-cells, and later released into the capillaries of the islets in response to appropriate stimuli. These capillaries release them into the portal vein that carries blood from the stomach, intestines, and pancreas to the liver. A healthy and a normal adult pancreas contains approximately 200 units of insulin. The average daily secretion of insulin into the circulation in healthy individuals ranges from 30 to 50 units (Nair et al., 2020).

Insulin is produced and stored in the body as a hexamer, which is inactive and very stable, but the active form is the monomer (Figure 1 left), and 6 monomers readily form 3 dimers (Figure 1 middle) that assemble into a hexamer (Figure 1 right) in the presence of zinc ions (Zn^{2+}) (Mirsky et al., 1963; Dunn, 2005). On the other hand, when insulin secreted from the *β*-cells, the zinc-insulin hexamers are diluted in the blood stream that results in the hexamers disassembling into monomers that is the active state of insulin (Hirsch et al., 2020). The hexamer is about 36,000 Da in size, and far more stable than the monomer, but the monomer is a much faster-reacting drug because diffusion rate is inversely related to particle size (Brange et al., 1990; Weiss et al., 2000).

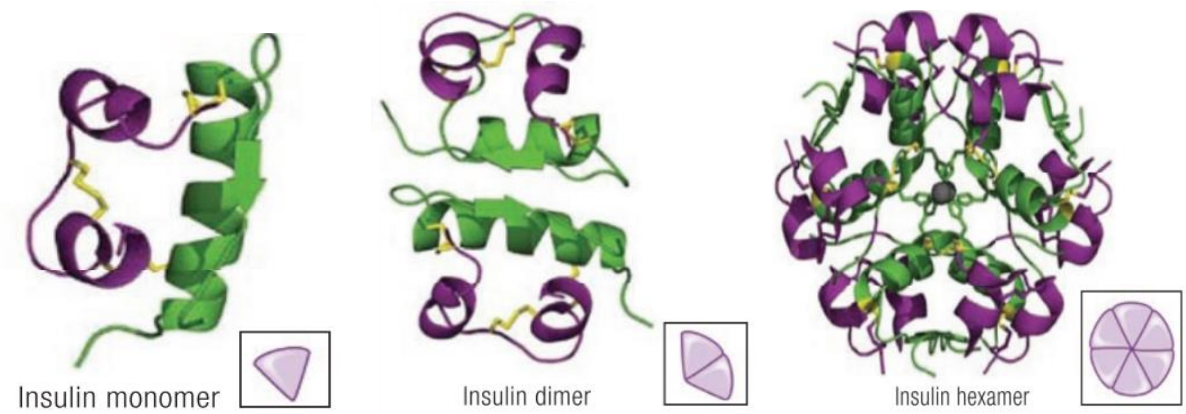

Figure 1. Structure of human insulin: monomer in left, dimer in middle, and hexamer in right

Source: Hirsch, et al. (2000).

7. Insulin Discovery

In 1869, a German medical student named Paul Langerhans (1847-1888), later became German pathologist, physiologist, and biologist; was studying the structure of the pancreas under a microscope, and had identified some unnoticed tissue clumps scattering within the pancreas. But he knew nothing about the significance of pancreatic islets. He called them "*little heaps of cells*", and was subsequently named after him "*Islets of Langerhans*" (Langerhans, 1869). In 1889, German physician and physiologist Oskar Minkowski (1858-1931) and German physician Joseph von Mering (1849-1908), who were conducting their famous experiment completely removing the pancreas from a dog (pancreatectomy), and producing severe and fatal diabetes (von Mering & Minkowski, 1890; Shah et al., 1997). In 1893, the French physician Emmanuel Hedon (1863-1933) performed total pancreatectomy on a dog and the dog developed diabetes only after the graft was removed that confirmed the internal secretion of the pancreas (Hedon, 1898). In 1893, French pathologist and histologist Gustave Edouard Laguesse (1861-1927) has suggested that pancreatic islet cells are involved in diabetes (Laguesse, 1893).

German physician George Ludwig Zuelzer (1870-1949) succeeded using pancreatic extracts on diabetic dogs. In 1906, he injected an extract called "Acomatol" into a dying diabetic patient who was in a coma. Initially, the patient showed improvement, but then suffered severe side effects and died when the supply of Acomatol was exhausted. In a total of seven patients he observed a marked reduction of glucose and acetone in the urine. But most patients suffered from severe side effects developing high fevers with tremors, sweating, and increased heart rate (Zuelzer, 1908; Tattersall, 2009). In 1915, biochemist Israel Simon Kleiner (1885-1966) studied pancreatic extracts at Rockefeller University. He demonstrated the blood sugar-lowering effect of intravenously administered pancreatic extracts in animal experiments. Kleiner was one of the first to demonstrate the effect of extracts from the pancreas on animals, causing hypoglycemia, which eventually helped lead to the discovery of insulin (Kleiner, 1919; Bliss, 1982).

Canadian medical scientist, physician, and painter Frederick Grant Banting (1891-1941) hypothesized that ligation of the pancreatic ducts would prevent contamination of pancreatic extracts by digestive enzymes. In 1921, the first peptide hormone insulin was discovered by Banting and American-Canadian medical scientist Charles Herbert Best (1899-1978), when they were working in the laboratory of Scottish biochemist and physiologist professor, diabetes expert, and head of the department of physiology, John James Richard Macleod (1876-1935) at the University of Toronto. They were the first to isolate insulin from dog pancreas through the surgery of pancreatic duct ligation. On August 3, 1921, Banting and Best's crude extracts from the pancreas of a dog first showed activity in reducing hyperglycaemia in a pancreatectomized dog (Hume, 2001; Banting & Best, 1922a).

Banting, an experience surgeon, met with Macleod for laboratory space, and Macleod granted him a small laboratory space, ten dogs for his experiments, fourth year physiology and biochemistry two students as research assistants, named Charles Best and Clark Noble. Finally, Best was appointed through a coin toss to work with Banting; and Macleod himself provided supervision and guidance (Quianzon & Cheikh, 2012). They have observed that removing the pancreas from a dog (pancreatectomy) developed diabetes and that intravenous injection with their pancreatic extract, which they named isletin that lowered the blood glucose. Later, in order to better adapt to an international audience, "Isletin" was replaced by the name "Insulin" (Cassier & Sinding, 2008; Chatterjee et al., 2013). Canadian biochemist James Bertram Collip (1892-1965) has used the purified pancreatic

extract and observes that blood glucose and glucosuria decreased, and ketonuria disappeared. He has developed a method for extraction and purification of substantial quantities of the hormone from bovine pancreatic tissue by changing the concentrations of slightly acidic alcohol solutions of chilled beef pancreas (Collip, 1923; Majumdar, 2001). In 1916, Romanian physiologist Nicolae Constantin Paulescu (1869-1931), who was working independently in the Medical School in Bucharest, Romania, succeeded in demonstrating in extensive experiments of the blood sugar-lowering and antiketogenic effect of an aqueous pancreatic extract. He named the antidiabetic principle "Pancreine" (Murray, 1971; Diema et al., 2021).

Banting decided to give the extract in humans. The first patient was a 14-year-old boy named Leonard Thomson (1908-1935), severely infected patient with type 1 diabetes, who had reached the brink of the grave. He weighted a mere 27kg, and urine was full of acetone and sugar, and breath was ketotic and waited for the inevitable end (Pratt, 1989). Activity was first shown in humans on January 11, 1922, when Thompson at Toronto General Hospital, Toronto, Canada, received an intravenous injection of a 15ml of pancreatic extract made by Banting and Best (Banting et al., 1922). The results were disappointing; and no clinical benefit was observed. The injection caused only slight reductions of glycemia and glycosuria, and had no effect on ketoacidosis; and the treatment was immediately discontinued (Joshi et al., 2007). Later, on January 23, 1922, an injection of a sufficiently purified insulin extract prepared by James B. Collip was tested on Thompson and the results were marked improvements in blood glucose and in urinary sugar and ketone levels. Glycaemia decreased from 520mg/dl to 120mg/dl, glycosuria dropped from 71g to 9g, and ketonuria disappeared; and within minutes Thompson started waking up from his coma, became brighter and was getting stronger (Collip, 1923; Li, 2003). Thompson recovered and then lived for 13 years more with the aid of insulin, and died due to broncho-pneumonia at the age of 27. In February 1922, the injections of the miraculous extract were administered to six patients (Banting & Best, 1922b).

The use of insulin has increased very rapidly and by November 1923, University of Toronto's Connaught Laboratories was producing 250,000 units of insulin per week. In 1922, Eli Lilly began to manufacture insulin from animal pancreas, but could not meet demand and potency varied up to 25% per lot (Rosenfeld, 2002). In 1923, Banting and Macleod were awarded the Nobel Prize in Physiology or Medicine for the discovery of life-saving insulin. Banting shared his half of the prize money with Best, while Macleod shared his half with Collip (Pickup & Williams, 2003; Diema et al., 2022). But Paulescu and Noble were officially excluded from the discovery of insulin (Rosenfeld, 2002). Five scientists are credited for the discovery of insulin, such as Frederick Banting, Charles Herbert Best, John J. R. Macleod, James Bertram Collip, and Nicolae Paulescu. Their contributions are tremendous in different areas of research, such as Banting's abilities as a surgeon, Best's enthusiasm as a student, Collip's abilities as a biochemist, and Macleod's guidance for the discovery of insulin (Rosenfeld, 2002).

8. Improvement of Insulin

Therapeutic insulin has evolved from a crude extract of animal pancreas to recombinant human insulin and insulin analogs. By the end of 1923, key developments of insulin in labs have performed, and the pharmaceutical industry has commercially produced insulin. There is an enormous change and innovation in the field of insulin therapy, such as isolation of insulin, purification and concentration of animal pancreatic extracts, duration of action, progression to human insulin, and modified insulin analogs made with recombinant DNA technology (Hirsch, et al., 2020).

At present various types of insulin are available, such as short-acting, long-acting, intermediate-acting, and rapid-acting. Initially, big and heavy reusable syringes with plungers, barrels, and long large-bore needles were used for insulin delivery, and these are sterilized by boiling to ensure efficient reuse. The first specialized syringe for insulin injection was manufactured by Becton Dickinson (BD) in 1924 (BD, 2019). Insulin was crystallized in 1926 by American biochemist and pharmacologist John Jacob Abel (1857-1938) (Abel, 1926). In 1936, Zinc-protamine insulin was developed by the Canadians David A. Scott and A. M. Fisher which presented a longer-acting insulin source (Scott & Fisher, 1938).

In 1946, Danish company Novo Nordisk developed Neutral Protamine Hagedorn (NPH or Isophane insulin) that was a combination of insulin and protamine in stoichiometric quantities, which was neutral insulin with longer duration of action that is used by injection under the skin once to twice a day (Owens, 1986; Gualandi-Signorini & Giorgi, 2001). In 1954, the first disposable glass syringe, the HypakTM (BD), was launched (Fry, 2012). In 1955, the British biochemist Frederick Sanger (1918-2013) has managed a full sequence of the bovine insulin and discovered its structure in terms of amino-acids (Stretton, 2002). In 1957, French physician Jean Sterne (1909-1997) for the first time used oral medications, such as metformin to treat diabetes patients (Bailey, 2017).

In 1963, Arnold H. Kadish who designed the first closed-loop insulin pump device that worked by providing continuous insulin to the body together with automatic blood glucose sensing. It is an artificial pancreas comprised of a large pump with an auto-analyzer that is operated to measure blood sugar with an on-off

servo-mechanism that controlled the pump function when blood sugar was outside normal ranges. Unfortunately, this device is poorly used due to its impracticality for daily use, with its size similar to “an army backpack” (Kadish, 1963; Alsaleh, 2010; Trevitt et al., 2015).

In 1965, first blood glucose test strip, the Dextrostix, is developed. It is a self-monitoring blood glucose (SMBG) system that plays a potential important role in the management of diabetes and in the reduction of risk of serious secondary clinical complications (Clarke & Foster, 2012). In 1969, British Chemist Dorothy Mary Crowfoot Hodgkin (1910-1994) determines the three-dimensional crystal structure of insulin using X-ray crystallography, after 35 years of study. She and her team solved the insulin structure, revealing the secondary, tertiary and quaternary structures (Adams et al., 1969; Glusker, 1994).

In 1975, fully synthetic insulin (CGP 12 831) was synthesized in the laboratories of Ciba-Geigy in Basel (Schlüter et al., 1981). In 1977, the radioimmunoassay (RIA) to measure insulin in the body was developed by Solomon Berson and Rosalyn Sussman Yalow at the Veterans Administration Hospital in the Bronx, New York. A RIA is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes (Berson & Yalow, 1959). In 1978 and 1979, American molecular biologist David V. Goeddel and his colleagues of the biotechnology firm Genentech use recombinant DNA techniques to produce synthetic human insulin using E. coli bacteria, and also produced human growth hormone, and human tissue plasminogen activator (tPA) for use in therapeutic medicine (Nielsen, 2013). The first biosynthetic human insulin product was approved in 1982, under the brand name Humulin R. The NovoPen was the first insulin pen that was launched by Novo Nordisk in 1985, which offer simpler, accurate, and convenient insulin delivery over syringes (Novo Nordisk, 2010).

9. Conclusions

From this study we have realized that before the discovery of insulin, physicians had no useful and skill tool to treat diabetes except the easily measurement of glucose in urine, and only insurance companies were benefited from this. The patients survive only few months to two years through the “starvation dieting”. Medical scientists Frederick Grant Banting and Charles Herbert Best worked in difficult conditions with determination, selflessness, and belief in success, and they finally succeeded through the discovery of the insulin, with the culmination of long waiting research on endocrine pancreas physiology. The discovery of the insulin is one of the most important medical discoveries in curative medicine. It is the result of the dedicated works of hundreds of researchers. In the 21st century, after 100 years of the discovery of insulin therapy, diabetes patients are gaining benefits continues use of insulin.

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