Research, Society and Development, v. 12, n. 6, e14712642090, 2023
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v12i6.42090

The Nobel Prize in Physiology 1977: A literature review of the Radioimmunoassay Technique

O Prêmio Nobel de Fisiologia de 1977: Uma revisão da literatura sobre a Técnica de Radioimunoensaio

El Premio Nobel de Fisiología 1977: Una revisión de la literatura acerca de la Técnica de Radioinmunoensayo

Received: 05/27/2023 | Revised: 06/09/2023 | Accepted: 06/10/2023 | Published: 06/15/2023

Matheus dos Santos do Nascimento Carvalho
ORCID: https://orcid.org/0000-0003-4907-1750
Universidade Estadual de Ciências da Saúde de Alagoas, Brazil
E-mail: matheus2029mpc@gmail.com
Adriano Santana de Luna Costa
ORCID: https://orcid.org/0000-0002-1711-777X
Universidade Estadual de Ciências da Saúde de Alagoas, Brazil
E-mail: adriano_uncisalmed@hotmail.com
Allan Vieira Almeida Santana
ORCID: https://orcid.org/0009-0009-4975-531X
Universidade Federal de Alagoas, Brazil
E-mail: allanavas88@gmail.com
Rodrigo Santana de Luna Batista
ORCID: https://orcid.org/0009-0007-8093-0820
Centro Universitário Tiradentes, Brazil
E-mail: rodrigobmmed@gmail.com
Fernando Pinto de Mesquita Neto
ORCID: https://orcid.org/0009-0008-6272-5601
Universidade de Brasília, Brazil
E-mail: fernandomesquita.neto@hotmail.com
Jefferson Cavalcante de Lima
ORCID: https://orcid.org/0000-0002-5494-0570
Universidade Estadual de Ciências da Saúde de Alagoas, Brazil
E-mail: jeffersoncmed@gmail.com
Adriane Borges Cabral
ORCID: https://orcid.org/0000-0002-4417-7559
Universidade Estadual de Ciências da Saúde de Alagoas, Brazil
E-mail: adriane.cabral@uncisal.edu.br

Abstract
The first study about radioimmunoassay was published in 1960 by 2 scientists, who created this new method to quantify human insulin, at a time when studies on diabetes and its pathophysiology were beginning to stand out. Years later, due to its strong relevance to the scientific world, such work was awarded the Nobel Prize in Physiology in 1977. Thus, the present study aims to expose the historical context of the discovery, the initial studies on diseases whose immunological origin was still unknown, the beginning of radioimmunoassay techniques and their potential benefits and effects for science. Therefore, a narrative review of the literature was carried out using the PubMed, Scielo and Google Scholar databases in order to find the theoretical bases that underlie the objective of the present study. The study revealed that through the use of radioisotopes for insulin labeling and the use of antibodies from animals, it was possible to quantify hormones at low blood concentrations, based on the graphic of the proportion between free marked insulin and insulin combined with antibodies. Such discovery was important to understand the pathophysiology of many diseases, although on the other hand it presents its risks because it involves radioactive material and because it is now in disuse due to the creation of methods such as ELISA. It is concluded that due to the historical and scientific importance of the RIA, this method proved to be worthy of the most renowned scientific award in the world.
Keywords: Radioimmunoassay; Nobel; Physiology; Insulin.

Resumo
O primeiro estudo sobre radioimunoensaio (RIA) foi publicado em 1960 por 2 cientistas, que criaram este novo método para quantificar a insulina humana, em uma época em que os estudos sobre a diabetes e a sua fisiopatologia começavam a se destacar. Anos mais tarde, por sua grande relevância para o meio científico, tal trabalho foi agraciado com o Prêmio

Nobel de Fisiologia em 1977. Assim, o presente estudo visa expor o contexto histórico da descoberta, os estudos iniciais sobre doenças cuja origem imunológica ainda era desconhecida, o início das técnicas de radioimunoensaio e seus potenciais benefícios e efeitos para a ciência. Para tanto, foi realizada uma revisão narrativa da literatura nas bases de dados PubMed, Scielo e Google Acadêmico, a fim de encontrar as bases teóricas que fundamentam o objetivo do presente estudo. O estudo revelou que através do uso de radioisótopos para marcação de insulina e o uso de anticorpos provenientes de animais, foi possível quantificar hormônios de baixas concentrações sanguíneas, baseado no gráfico da proporção entre insulina marcada livre e insulina combinada com anticorpos Essa invenção se tornou ferramenta no entendimento da fisiopatologia de muitas outras doenças, embora por outro lado apresenta seus riscos por envolver material radioativo e por hoje estar em desuso devido a criação de métodos como o ELISA. Conclui-se que devido a importância histórica e científica do RIA, tal método mostrou ser digno do prêmio de maior renome científico do mundo.
Palavras-chave: Radioimunoensaio; Nobel; Fisiologia; Insulina.

Resumen
El primer estudio sobre radioinmunoensayo fue publicado en 1960 por 2 científicos, que crearon este nuevo método para cuantificar la insulina humana, en un momento en que empezaban a destacar los estudios sobre la diabetes y su fisiopatología. Años más tarde, por su gran relevancia para la comunidad científica, este trabajo fue galardonado con el Premio Nobel de Fisiología en 1977. Así, el presente estudio pretende exponer el contexto histórico del descubrimiento, los estudios iniciales sobre enfermedades cuyo origen inmunológico aún era desconocido, el comienzo de las técnicas de radioinmunoensayo y sus posibles beneficios y efectos para la ciencia. Así, se realizó una revisión narrativa de la literatura en las bases de datos PubMed, Scielo y Google Scholar, con el fin de encontrar las bases teóricas que sustentan el objetivo del presente estudio. El estudio reveló que mediante el uso de radioisótopos para el marcaje de insulina y el uso de anticuerpos de animales, fue posible cuantificar hormonas a bajas concentraciones en sangre, con base en el gráfico de la proporción entre insulina libre marcada e insulina combinada con anticuerpos. Esta descoberta ayudó a comprender la fisiopatología de muchas enfermedades, aunque por otro lado presenta sus riesgos por tratarse de material radiactivo y por estar en desuso debido a la creación de métodos como ELISA. Se concluye que debido a la importancia histórica y científica del RIA, este método resultó ser merecedor del premio científico más renombrado del mundo.
Palabras clave: Radioinmunoensayo; Nobel; Fisiología; Insulina.

1. Introduction

In 1960, an article by Rosalyn Sussman Yalow and Solomon Aaron Benson was published, in which they described for the first time a new method for the quantification of hormones, which due to its low concentrations in the blood, estimating hormone levels was a real challenge. With this article, they have won the 1977 Nobel Prize, with the proposal of the radioimmunoassay (RIA) as a way of accounting serum insulin in humans. Their studies were based on the application of antibodies and their action in binding to insulin. A mixture of insulin labeled with the radioisotope Iodine-131 was applied, and this insulin, with previously known concentrations, would compete with serum insulins whose concentration was unknown (Zárate & Manuel, 2011; Kahn & Roth, 2012).

After the balance between free insulin and antibody-bound insulin got established through chromium electrophoresis, these components are separated and the ratio of insulin labeled with Iodine-131 bound to antibody and free labeled insulin would be inversely proportional to the concentration of unlabeled insulin, that is, the insulin wanted to know the concentration. Such explanation is based on the fact that with more serum insulin, greater competition with insulin marked by the antibody site, and therefore lower the B/F ratio. From this, a graphic is formed and will detail the insulin concentrations(Yalow & Berson, 1960; Zárate & Manuel, 2011).

Such method was enough to prove once again the difference between diabetes mellitus I and II, in which type II is marked by high insulin values, as a compensation for the lower sensitivity of tissues to glucose, while type I diabetes is characterized by low levels of production of this hormone, which requires external insulin replacement to control serum glycemia(Yalow & Berson, 1960; Kahn & Roth, 2004; Zárate & Manuel, 2011).

Finally, with this method, other hormones could be measured, aiding in diagnosis, helping to understand many pathologies and being the beginning for the development of new efficient forms of endocrine measurement (Kahn & Roth, 2004). Furthermore, this study aimed to expose and describe the importance of the award Nobel Prize in 1977, evaluating the historical

context of the discovery, the initial studies on diseases, whose immunological origin was still unknown before the RIA, the beginning of radioimmunoassay techniques and their potential benefits and effects for science.

2. Methodology

The present study is a narrative review carried out (Pereira et al., 2018) with a descriptive character about the 1977 Nobel Prize in Physiology. The review included scientific articles, monographs, theses and dissertations published and available in the PubMed, Scielo and Google Scholar without publication date restriction. It was selected those studies that have demonstrated the historical aspects, scientific ideology that inspired the Nobel-winning idea and the positive and negative points of the discovery for science. The descriptors "Nobel Physiology 1977", "Rosalyn S. Yalow", "Solomon A. Benson", "Radioimmunoassay" and "ELISA" using the Boolean operator "AND" were used. Studies in English, Portuguese and Spanish were included and those that did not present an abstract or title according to the theme, as well as those with poor methodology, letters to the editor and opinion articles were excluded.

3. Results and Discussion

3.1 Historical aspects and authors

Rosalyn Sussman Yalow was born on July 19, 1921 in New York, grew up inspired by Marie Currie, famous for her work on radioactivity, and with a great interest in science from her young age. She has studied at Hunter College, where she got his admiration for physics, especially nuclear physics. Years later, she landed a position as an assistant professor of physics at the University of Illinois. During her stay, she was the only woman out of 400 men, in a period when women did not have the same opportunities as nowadays (Zárate & Manuel, 2011; Kahn & Roth, 2012).

She has grown in the period of the events of the Second World War, a war that increased investments in the nuclear sector, through studies of nuclear fission, bombs or even through the application of radioisotopes in a various areas of science. It was at the University of Illinois that Rosalyn Yalow met Aaron Yalow, a post-doctoral fellow in physics, whom she would marry in 1943. Furthermore, with the advent of world war, she had to organize herself as a wife, teacher, and housekeeper in times of scarcity and economic crises arising from the military confrontation. In 1945, she began her doctorate in nuclear physics (Zárate & Manuel, 2011; Kahn & Roth, 2012).

Years later, she began to do clinical research involving the use of radioisotopes, when she joined the Radiotherapy Service at the Bronx Veterans Administration Hospital. This period, she met Dr. Solomon A. Berson, whose scientific partnership supported the research that would lead to the Nobel Prize. They especially applied nuclear knowledge and the use of iodine as a marker, in order to finally create an efficient method for quantifying circulating insulin in the blood (Kahn & Roth, 2012).

Solomon Aaron Berson was born on april 22, 1918, in the city of New York, and being a russian immigrant's son, he showed talent from an early age. He graduated from the City College of New York, but was rejected by many medical universities before finally being accepted at the New York School of Medicine. Later, he served as a resident at the Bronx Veterans Administration Hospital, where he met his future research partner, Rosalyn Yalow, with whom he would carry out his studies with radioisotopes and the creation of the radioimmunoassay (RIA) method (Friedman, 2002).

In their studies, Yalow and Berson analyzed insulin quantification, possibly following Dr. Arthur Bauman indications, and probably for some emotional factor, as long as Yalow's husband was diabetic. However, before carrying out the studies with insulin labeling, they had already marked electrolytes and red blood cells to study the behavior and reaction of these components in the human body (Friedman, 2002).

With the progress of these studies, they made important discoveries in immunology, until the day that Arthur Bauman and Marcus Rothschild applied the RIA method clinically for the first time. With these experiments from Yalow and Berson, the

hypothesis that insulin could be considered an antigen and would stimulate the production of antibodies arises. Such hypothesis, which was very controversial at the time, caused their article to be denied for publication by the famous scientific journals Science and The Journal of Clinical Investigation (Friedman, 2002; Kahn & Roth, 2004).

In 1968, Berson was transferred from the laboratory to serve as president of the Department of Medicine at the Mount Sinai School of Medicine. Unfortunately, years later Berson got dead, preventing him from enjoying the 1977 Nobel Prize, along with Yalow. The history of this duo certainly contributed scientifically for the world and brought inspiration for many studies and researchers. It has also shown that Yalow, in a time still marked by little female participation in the scientific world she achieved great goals, and Berson, being the son of an immigrant and rejected by several medical schools, still persisted in his dream (Friedman, 2002; Kahn & Roth, 2012; Minella, 2017)

3.2 Contextualization of the Discovery of Radioimmunoassay

Before the innovation brought by the Nobel Prize winners in 1977, there were many difficulties in measuring the concentration of substances in the human body, as well as certain diseases that were still little known, such as diabetes mellitus. One of the major problems related to measurement was precisely the low concentration of certain hormones in blood plasma and insensitive methods for detecting such low concentrations (Kahn & Roth, 2004; Wheeler, 2013).

Measurement methods at the time were performed with hypophysectomized or adrenalectomized rats, to ensure greater sensitivity to insulin, however low concentrations used to difficult the measures, so that hormone quantifications could only be performed with the extraction of large amounts of blood, which would be unfeasible (Kahn & Roth, 2004; Wheeler, 2013).

3.2.1 Initial studies about insulin

In the 20th century, a hormone that drew a lot of attention was precisely insulin. In 1921, the isolation of insulin was carried out by Banting and collaborators, in which they carried out procedures to separate the islet of Langerhans from the exocrine part of the pancreas, in order to obtain the hormonal substrate. This method was based on a surgical technique that consisted of closing the pancreatic duct with consequent degeneration of the exocrine pancreas, thus managing to collect only the islet of Langerhans, responsible to produce insulin (Vecchio et al.,2018).

With this discovery, exogenous insulin was applied as a treatment for the first time in the diabetic patient Leonard Thompson, obtaining promising results in glycemic control, which, in the case of this patient, reduced from 520 mg / dl to 120 mg / dl. Subsequently, Frederick Sanger was able to sequence the constituent amino acids of bovine insulin, discovering that such hormone is formed by 2 chains (A and B) linked by a disulfide bridge, the A chain being composed of 21 amino acids and the B chain by 30 (Stretton, 2002; Guyton & Hall, 2017; Vecchio et al., 2018).

It is important to emphasize that insulin is a hormone released freely into the plasma by the endocrine portion of the pancreas and that it usually has a half-life of 6 minutes, and about 15 minutes it is practically disintegrated. It has a portion that binds to a cellular receptor, such receptor has 2 alpha subunits external to the cell and 2 beta subunits that cross the membrane and have a portion internal to the cell (Guyton & Hall, 2017; Rahman et al, 2021).

With the binding of insulin to alpha subunits, its non-binding portion is disintegrated by insulinase, however, this binding portion causes phosphorylation of the beta subunit, followed by activation of tyrosine kinase, which will phosphorylate and activate several proteins, especially from a class of substrates of insulin receptors (IRS). Therefore, with such phosphorylations, protein channels for glucose are transported to the cell surface, thus resulting in increased permeability of glucose in tissues and consequent reduction of serum glucose (Guyton & Hall, 2017; Rahman et al, 2021).

3.2.2 Discovery of the types of D*iabetes mellitus* and diagnostic challenges

Years later, Sir Harold Himsworth discovered the 2 types of diabetes: I and II. Basically, diabetes I is characterized by a deficiency in the production of insulin. It may be involved with the HLA gene system, with prevalence of DQ2 and DQ8 alleles. Such alleles influence the formation of MHC class II by macrophages, which will support the self-immune action from CD4+ T lymphocytes against the endocrine portion of pancreas tissue (Piya & Michels, 2012; Banday et al., 2020).

There are evidences of autoantibodies in patients with type I diabetes, which act by destroying the Beta cells of the islets of Langerhans (endocrine portion of the pancreas). They are antibodies against markers such as GAD65, ICA512, ZnT8 or even against insulin itself. Such actions cause the reduction of serum insulin, causing problems such as hyperglycemia and glycosuria. Basically, in the analyzes with non-obese diabetic rats (NOD) after T cells recognize the self-antigen presented in MHC molecules, these T cells, through molecular similarity, recognize amino acid sequences of the B chain of insulin as an antigen. From this reaction, an inflammatory and destructive process of the Beta cells of the Islet of Langerhans begins. These activated T cells stimulate the B lymphocytes to produce antibodies against insulin, and in the destruction of the Beta cells of the islet, the phenomenon of "epitope spreading" occurs, in which more self-antigens are released and new immune responses are being triggered (Piya & Michels, 2012; Banday et al., 2020).

There is also type II diabetes mellitus, whose main causes are genetic or a sedentary lifestyle and inadequate diet that leads to obesity. Obesity is characterized by the increase of fat tissue in the body. The accumulation of fat tissue tends to cause many problems that will act to reduce the sensitivity of hepatocytes, adipocytes and muscle tissue to insulin produced by the islets of the pancreas (Banday et al., 2020).

It is known that much of this resistance comes biochemically from a reduced tyrosine phosphorylation of the insulin receptor and IRS proteins in peripheral tissues, such conditions interfere with a greater uptake of GLUT-4 (glucose transporter) to the cell surface, and consequently in a tissue resistance to the effect of insulin in stimulating absorption of glucose from the blood. At first, in obesity, adipocytes tend to reduce the production of the hormones leptin and adiponectin, which would act by increasing the sensitivity of cells to insulin (Abbas & Aster, 2016; Galicia-Garcia et al., 2020).

There is also an increase in circulating fatty acids, mainly due to tissue deficiency in inhibiting the action of lipoprotein protease. The increase in the action of this protease will promote high levels of free fatty acids, which will overload the pathways of oxidation of intracellular fatty acids, leading to the formation of toxic compounds that will inhibit the signaling pathways of the insulin receptor pathway. Such problem in signaling can affect the hepatocytes, that perform gluconeogenesis, due to the fact that the increase in fatty acids and their influence on signaling will make the enzyme carboxykinase phosphoenolpyruvate stimulate gluconeogenesis, which will further contribute to hyperglycemia (Abbas & Aster, 2016; Galicia-Garcia et al., 2020).

Finally, excess nutrients (glucose and fatty acids) can create an environment conducive to inflammation. In this way, there is production of cytokines, such as IL-1Beta, which will mediate the secretion of pro-inflammatory cytokines, such as IL-1, which will act on insulin receptors, promoting tissue resistance to this hormone. In addition, the islet beta cells begin to produce higher concentrations of insulin in order to compensate the resulting hyperglycemia, however, in longer effects, lipotoxicity, glucotoxicity and amyloid deposition cause a depletion of the insulin release function by such cells, which ends up corroborating with higher blood glucose levels (Abbas & Aster, 2016; Galicia-Garcia et al., 2020).

Therefore, the hormone insulin acts by stimulating the entry of glucose into the cell, thus controlling blood glucose, and one of the major differences between types of diabetes mellitus I and II is the concentration of insulin in the blood, the measure of the concentration of hormones has become essential for diagnosing many endocrine disorders (Kahn & Roth, 2004).

3.2.3 Beginning of studies and techniques on radioimmunoassay

Quantifying the hormone concentration has always been challenging, precisely because of its low concentrations in the blood. Thus, the pair of scientists Yalow and Berson published in 1960 the article called "IMMUNOASSAY OF ENDOGENOUS PLASMA INSULIN IN MAN", in which they described the method of measuring and quantifying hormones with great sensitivity and precision called radioimmunoassay (RIA).

In their initial method, they injected bovine protamine zinc insulin subcutaneously into guinea pigs until antibodies against insulin developed in their bodies. Subsequently, they made a preparation of I-131 with insulin, in order to mark them with this radioisotope. For the preparation, they used a mixture containing chloroform (CCl^4), HCl, KI, I-131 and $NaNO^2$, this mixture is properly capped, in order to avoid loss of I-131 in the atmosphere and carried out in specific concentrations, in order to avoid an activity strong enough to destroy insulin (Yalow & Berson, 1960).

From the mixture between chloroform and iodine, bovine crystallized insulin was added, which is more practical to be studied than those from humans. In this process, care must be taken with the damage that radioactive iodine can cause to insulin, so that the contact between them must be brief, together with the use of adsorbents that will retain excess iodine, aiming to guarantee that in the end produce 3 to 5 µg of insulin labeled with 1.0 to 2.0 mc Iodine-131. This procedure faces certain problems, such as the fact that about 4 to 6% of the components of the solution are damaged by radiation. Such damaged components need to be removed, and the iodine-131-labeled insulin solution (insulin-I-131) needs to be purified. Such separation is done through a cellulose column, which will adsorb the undamaged insulin and allow its purification (Yalow & Berson, 1960).

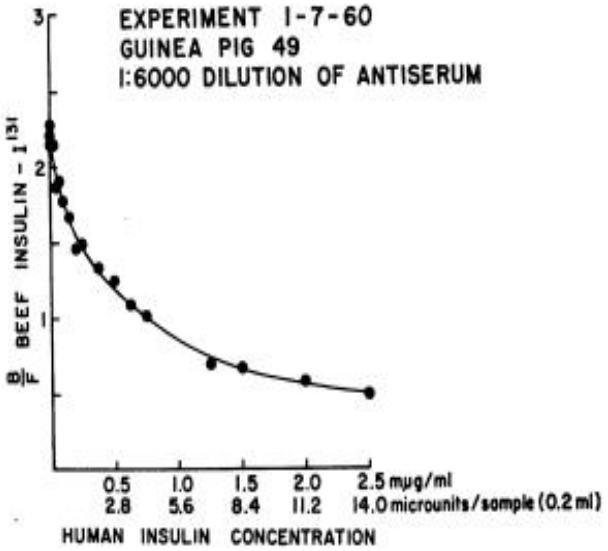
3.2.4 Understanding the concept of radioimmunoassay

Basically, the radioimmunoassay is theorized in the fact that the collected sample of "human insulin" is recognized by "anti-insulin antibodies" from guinea pigs, but "human insulin" will compete for binding with the antibody against "crystallized bovine insulin–131", which is easier to obtain. In this mixture, the antibodies will bind to both types of insulin and a portion of "free insulin" will remain that has not bound. After that, chromate electrophoresis is applied, which will separate the "bound insulin" from the "non-bound insulin to antibody" (Yalow & Berson, 1960; Blumenthal,2009).

The "bound insulins" will be attracted by the anode, which will also attract the inter-beta-gamma-globulins, and the "unbound insulins" will remain adsorbed in the place of origin. Thus, the ratio between "bound insulin-I-131 to the antibody" and "free insulin-I-131" is quantified (B/F ratio: Bound insulin/Free insulin), such ratio is a function of the concentration of "human insulin bound to the antibody". So, the more human insulin exists, there would be more competition for the antibody site, and the number of "bound insulin-I-131" will be lower. For example, Yalow and Berson stipulated that the presence of 15µg U/ml human insulin was sufficient to cause a 50% reduction in the B/F ratio (Yalow & Berson, 1960; Blumenthal,2009).

In their study, they defined 2 preparations containing "human insulin" in order to make the standard curve. To insulin (0.05 to 5.0 mug/ml) was added a standard solution from 0.05 to 0.15 mug/ml "bovine insulin-I-131" and the antibody. The mixture was refrigerated at 4°C for 4 days, enough time for the insulin-antibody reactions to reach equilibrium. Therefore, a chromate electrophoresis is performed for 1.5 hours, which is enough time to separate the "insulin-I-131 bound to the antibody" from the "unbound insulin" (Yalow & Berson, 1960).

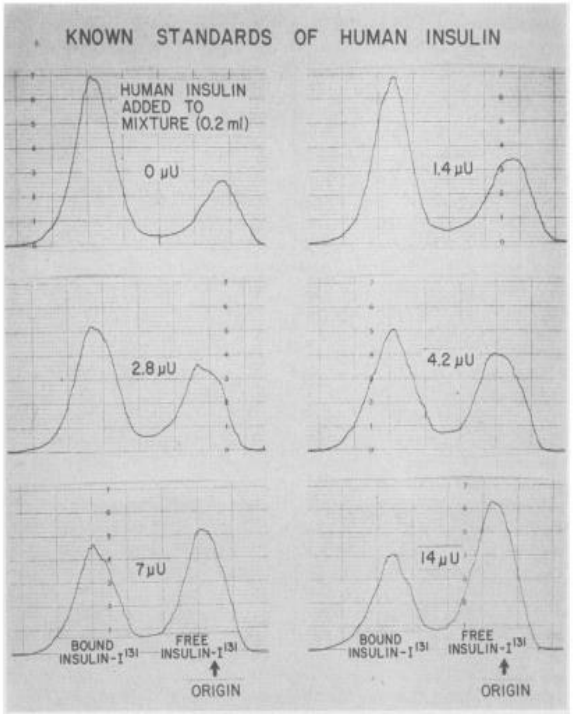
Figure 1 - B/F x Human insulin concentration curve published in the article "IMMUNOASSAY OF ENDOGENOUS PLASMA INSULIN IN MAN"



Source: Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest. 1960; 39(7): 1157–75.

From the results obtained, a B/F (Bound insulin/Free insulin) curve was made (Figure 1) as a function of the concentration of "human insulin" added, with the necessary corrections and considerations that 3 to 6% of the insulin was damaged by the radioactive effect. It is important to notice that B/F is inversely proportional to the human insulin.

Figure 2 - Representation of the graphic shown in the article "IMMUNOASSAY OF ENDOGENOUS PLASMA INSULIN IN MAN" of the distribution of peaks of labeled insulin linked to antibody (Bound insulin - I-131) and free insulin-I-131 (Free insulin-I-131) according to the concentration of insulin in μU.



Source: Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest. 1960; 39(7): 1157–75.

When it is noticed the graphics made by the authors (Figure 2), for each increase in the level of "human insulin", there is a disproportion in the peaks of "bound insulin-I-131" and "free insulin-I-131", in a way that concentration of "free insulin-I-131" becomes higher, and the B/F ratio decreases, that is, "bound insulin-I-131" peaks fall and "free insulin-I-131" peaks increase (Yalow & Berson, 1960).

So, the procedures for determining human insulin concentration can be performed following the same methods explained above, however the results obtained will be based on the standard curve created by them, which establishes the B/F ratio as a function of insulin concentration. In their article, they used this method to evaluate diabetic and non-diabetic patients, and excluded those patients who were previously treated with insulin, due to the fact that these patients would have naturally created antibodies against insulin, which would interfere in the results of the study (Yalow & Berson, 1960).

Thus, in their conclusions, they noticed the great sensitivity of the new method created, which would be able to detect insulin concentrations fewer than 1 μU. After that, this method was expanded and used to detect other hormones, which has helped in the diagnosis of many diseases. Nevertheless, the RIA study has brought some controversies, one of them, which is worth mentioning, was the difference in the values found for insulin immunoreactive through RIA method before the high values of insulin bioactivity in the organism, which led to the hypothesis that perhaps not all insulin would react with "anti-insulin antibodies". This would be explained years later through the discovery of IGF (Insulin-like growth factor), a compound responsible for the high values of insulin bioactivity, but without influence in diabetes pathogenesis (Yalow & Berson, 1960; Kahn & Roth, 2004).

3.3 Benefits Brought by the Discovery

Initially with the discovery, in the period of the cold war, in which nuclear science was only aimed at military use, the study by Yalow and Berson with radioisotopes certainly showed that nuclear science deserved to be respected and could be very useful for medical applications and, above all, preservation of life. With the RIA technique, it became possible to accurately and sensitively quantify the concentration of circulating hormones, thus facilitating the diagnosis of many pathologies. Later, with this method, other hormones began to be quantified and diagnoses of other endocrine diseases began to be detected (Kahn & Roth, 2004).

The radioimmunoassay method created by Yalow and Berson managed to quantify insulin and later could inspire new studies to quantify other endocrine components, such as PTH, the hormone responsible for increasing serious calcemia, in a partnership carried out with Gerald Aurbach and John Potts, and also the quantification of the hormone GH, produced by the anterior pituitary, which helped in the understanding of several pathologies and endocrine disorders (Friedman, 2002).

Currently, the radioimmunoassay technique is also performed with certain adaptations in a range of areas of health, for example, there are methods of quantifying hypocretin and thyroid hormones, in order to respectively diagnose type I narcolepsy (sleep disorder characterized by reduced levels of hypocretin) and thyroid disorders. In particular on type I narcolepsy, the method is based on the collection of cerebrospinal fluid (CSF) (containing hypocretin), such a sample is inserted into an environment with anti-hypocretin antibodies and radioactive (I-125) labeled hypocretin-1, which will compete with the CSF hypocretin by binding to the antibody, based on the radioactive concentration, the value of hypocretin is estimated, in a similar logic to the RIA with the measurement of insulin (Hoeven et al., 2022; Wu, 2022).

Furthermore, the use of antibodies as a way of measuring the concentration of some substrate was the beginning for new discoveries, one of them has began when Yalow and Berson in their studies noticed certain alterations in insulin levels, which led many scientists at the time to believe that not all insulin had the ability to bind to the antibody. Froesch & Zapf (1985) identified molecules called IGF-1 and IGF-2, which would act as cell growth factors, although they have a chemical structure similar to insulin.

With the invention of the radioimmunoassay, researches in this field were opened and more precise and effective methods of quantification were created, such as the ELISA method, a method increasingly used in laboratories, used to quantify both antigen and antibody. The first form of ELISA was the direct form, in which the patient's antigen of unknown concentration is searched in the microtiter plate (containing antibodies specific to the antigen to be studied), and then antibodies labeled with enzymes are added, which would later react with added substrates causing color alteration, and through the spectrophotometry it would be possible to measure and thus quantify the researched antigen (Sakamoto et al., 2018; Lin, 2015).

In this way, new ELISA methods have been created, such as the competitive and indirect form. The indirect form, per example, investigates the specific antibody of the patient's sample which is added to the container containing a specific antigen in the microtiter plate, which will be the binding site of the antibody. Subsequently, a second enzyme-labeled antibody is added that will also bind to the Fc fraction of the researched antibody. So, the enzyme in front of a chromogenic substrate will emit and generate color changes that will be measured and will show the concentration of the analyzed substance (Lin, 2015;Sakamoto et al., 2018; Alhajj & Farhana, 2023).

3.4 Possible Negative Effects on the Practical Application of the Discovery

As negative points, it is noticeable that compared to current methods, the radioimmunoassay method still has some problems. One of them involves the use of radioisotopes for the preparation of insulin, especially Iodine-131. The use of radioisotopes requires certain care in their preparation and handling, in view of their carcinogenic and mutagenic effects on the body. With the radioimmunoassay, the handling of radioisotopes has probably increased, which also increases the risk of accidents in the laboratory. Another proof of the danger of Iodine-131 is that, although it can be used in the treatment of hyperthyroidism, and even if the doses are controlled, people who have undergone this treatment need to take certain precautions, such as avoid contact with children and pregnant women, and keep the use of disposable objects (Greenlee et al., 2011).

When compared to current hormone quantification methods, radioimmunoassay has some disadvantages, especially when compared to ELISA. The radioimmunoassay has a higher cost, the shelf life of the reagents is shorter and there's the danger in manipulating radioactive products. Another problem with radiation is that a short percentage of insulin is degraded in RIA method, which implies a slight reduction in the accuracy of hormone counting, a reduction that will need to be compensated when reproducing the B/F x insulin graphic (Yalow & Berson, 1960; WHO, 1976)

4. Conclusion

To conclude, the radioimmunoassay was a very significant invention for science and for the world. Such project was a great inspiration, mainly because their authors were respectively a woman and an immigrant, in a time marked by strong discrimination, demonstrating the impetus and dedication of those involved. In addition, their innovative method could help in understanding the pathophysiology of many diseases, provide new forms of diagnosis, quantify many hormonal components and be the basis for the advancement of technology, such as the different types of ELISA methods currently available. Besides that, the RIA still is an inspiration for new and future methods that certainly contribute to the science advance. For all their historical and scientific legacy, Yalow and Berson certainly proved to be inspiring and worthy of the highest scientific award in the world.

References

Abbas, A. K. & Aster, J. C. (2016). Robbins & Cotran Patologia - Bases Patológicas das Doenças (9a ed.), Elsevier.

Alhajj, M. & Farhana A. (2023). Enzyme Linked Immunosorbent Assay. StatPearl [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK555922/

Banday, M. Z., Sameer, A. S. & Nissan, S. (2020). Pathophysiology of diabetes: An overview. *Avicenna J Med*., 10 (4), 174–188.

Blumenthal, S. (2009). The insulin immunoassay after 50 years: a reassessment. *Perspect Biol Med*., 52 (3), 343-354.

Friedman, A. (2002). Remembrance: The Berson and Yalow Saga. *The Journal of Clinical Endocrinology & Metabolism*., 87(5), 1925-1928.

Froesch, E. R. & Zapf, J. (1985). Insulin-like growth factors and insulin: comparative aspects. *Diabetologia*, 28, 485-493.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H. & Martin,C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci*., 21 (17), 6275.

Greenlee, C., Burmeister, L. A., Butler, R.S., Edinboro, C. H., Morrison, S. M. & Milas, M. (2011). Current safety practices relating to I-131 administration for diseases of the thyroid: a survey of physicians and allied practitioners. *Thyroid*., 21(2), 151-160.

Guyton, A. C. & Hall, J. E. (2017). Livro Tratado de Fisiologia Médica. (13a ed.), Elsevier.

Hoeven, A. E., Waaij, K., Bijlenga, D., Roelandse, F. W., Overeem, S., Bakker, J. A., Fronczek, R. & Lammers, G. J. (2022). Hypocretin-1 measurements in cerebrospinal fluid using radioimmunoassay: within and between assay reliability and limit of quantification. *Sleep*., 45(7).

Kahn, C. R. & Roth, J. (2004). Berson, Yalow, and the JCI: the agony and the ecstasy. *J Clin Invest*., 114(8), 1051–1054.

Kahn, C.R. & Roth, J. (2012). Rosalyn Sussman Yalow (1921–2011). *Proc Natl Acad Sci U S A*., 109 (3), 669–670.

Lin, A. V. (2015). Direct ELISA. *Methods Mol Biol*., 1318:61-7.

Minella, L. S. (2017). No trono da ciência I: Mulheres no Nobel da Fisiologia ou Medicina (1947-1988). *Cad. Pesqui*, 47(163), 70-93.

Pereira, A. S., Shitsuka, D. M., Parreira, F. J. & Shitsuka, R. (2018). Methodology of scientific research. UFSM https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf?sequence=1

Piya, A. & Michels, A. W. (2012). Understanding the Immunology of Type 1 Diabetes— An Overview of Current Knowledge and Perspectives for the Future. *US Endocrinology.*, 8(1), 70-74.

Rahman, S., Hossain, K. S., Das, S., Kundu, S., Adegoke, E. O., Rahman, A., Hannan, A., Uddin, J. & Pang, M. (2021). Role of Insulin in Health and Disease: An Update. *Int J Mol Sc*i., 22 (12), 6403.

Sakamoto, S., Putalun, W., Vimolmangkang, S., Phoolcharoen, W., Shoyama, Y., Tanaka, H. & Morimoto, S. (2018). Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. *J Nat Med*., 72 (1), 32–42.

Stretton, A. O. (2002). The first sequence: Fred Sanger and insulin. *Genetics*., 162 (2), 527-532.

Vecchio, I., Tornali, C., Bragazzi, N. L. & Martini, M. (2018). The Discovery of Insulin: An Important Milestone in the History of Medicine. *Front Endocrinol (Lausanne)*., 9: 613.

Wheeler, M. J. (2013). A short history of hormone measurement. *Methods Mol Biol*., 1065, 1-6.

WHO. (1976). The enzyme-linked immunosorbent assay (ELISA). *Bull World Health Organ*., 54 (2), 129–139.

Wu, Z. (2022). Effect of Radioimmunoassay on Accuracy of Thyroid Hormone Detection. *Contrast Media Mol Imaging*. 2022: 9206079.

Yalow, R. S. & Berson, S. A. (1960). Immunoassay of endogenous plasma insulin in man. *J Clin Invest.*, 39 (7), 1157–1175.

Zárate, A. & Manuel, L. (2011). Development of radioimmunoassay and its consequences in the medical research. A tribute to Rosalyn Yallow. *Rev Med Inst Mex Seguro Soc*., 49 (5), 465-468.