

# THE HISTORY OF BIOCHEMISTRY

UDC 616.07+616.379-008.64

doi: <https://doi.org/10.15407/ubj96.06.082>

## RADIOIMMUNOASSAY AND REVOLUTION IN MEDICAL INVESTIGATION: NOBEL PRIZE WINNER IN PHYSIOLOGY OR MEDICINE (1977) ROSALYN YALOW – SCIENTIST WITH A FIGHTING SPIRIT

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**Received:** 28 October 2024; **Revised:** 18 November 2024; **Accepted:** 21 November 2024

*If women are to start moving toward the goal, we must believe in ourselves or no one else will believe in us... Women in science should not ask for reverse discrimination, but simply for equality of opportunity - so that those of us who wish can reach for the stars.*

Rosalyn Yalow

*An intelligent but poor New York City girl, whom her parents saw in the future only as a teacher at best, Rosalyn Yalow after finishing school in 1937 went to all-female Hunter College and was the first woman with a degree in physics at a time when being a woman, much less a Jewish woman, was a severe barrier to success. Although she was called somewhat aggressive and high-brow she became the first woman student in the physics department at the University of Illinois, the only woman among 400 members of the College of Engineering faculty and after receiving Ph. D the only women engineer at the Bronx Telecommunications Laboratory. By 1950, she established a radioisotope laboratory in the Bronx Veterans Administration Hospital, one of the first in the United States. In collaboration with an M.D. Sol Berson, a research partnership with whom lasted for 22 years, Yalow investigated the metabolism of <sup>131</sup>I-labeled insulin in diabetic patients and not only demonstrated for the first time the production of antibodies against such a small protein but also developed the breakthrough radioimmunoassay (RIA) to measure insulin concentration in patient's blood with no radioactivity entered a body. This revolutionary technique began to be widely used around the world for measuring a variety of biological substances. In 1977 R. Yalow was awarded the Nobel Prize for Physiology or Medicine "for developing radioimmunoassays of peptide hormones." The review describes the scientific career and life path of this extraordinary woman.*

**Key words:** Rosalyn Yalow, radioimmunoassay, insulin, Nobel Prize, ELIZA.

Rosalyn Sussman Yalow, American medical physicist, sometimes characterized as "Madame Curie of the Bronx," was born on July 19, 1921 in the Bronx, New York City as the only daughter and second child of a lower middle-class Jewish parents. Her father, Simon Sussman, of Russian descent, was born on the Lower East Side of New York, the Melting Pot for Eastern European immigrants. Her mother, Clara (née Zipper), had emigrated to the United States from Germany at the age of four. Like many other Jewish immigrants, nei-

ther of parents had any education beyond elementary school, but they recognized the value of higher education for their children as a mean of social promotion [1].

Rosalyn was a stubborn, goal-oriented child, and no one could have deflected her from the path she had taken. She went to Walton High, an all-girl school in the Bronx, excelled in all academic subjects and showed an extraordinary talent in studying mathematics and chemistry. But the mid-1930s was a thrilling time when physics, especially

nuclear physics, was developing rapidly and Yalow was intrigued. She was determined to get a higher education and after graduating in 1937 at age fifteen from Walton School went directly to Hunter College, a highly competitive, tuition-free all-female college. Her parents believed their daughter could become a teacher like other college-educated young ladies. But instead, Rosalyn wanted to be a physicist [2, 3].

Rosalyn Yalow wrote: “When I was in college, nuclear physics was the most exciting field in the world. It seemed as if every major experiment brought a Nobel Prize. I was hanging from the rafters in a physics lecture room at Columbia University when Enrico Fermi gave a colloquium in January 1939 on the newly discovered nuclear fission – which has resulted not only in the terror and threat of nuclear warfare but also in the ready availability of radioisotopes for medical investigation”[4].

In 1941 Yalow graduated *magna cum laude* from Hunter College with a B. A. in chemistry and physics, winning high scholastic distinction and being elected to membership in a national student Phi Beta Kappa Honor Society founded in 1776 [5].

The first woman to graduate from Hunter College with a degree in physics, she became a physicist at a time when being a woman was a serious barrier to success. Her physics professor described her as a brilliant, but “very aggressive” and “as cold-blooded as possible” student in the sense that Yalow’s ambition was inappropriate a woman [6].

However, these aspects of her character, were necessary for Yalow to progress. She was excited about achieving a career in physics and was the perfect candidate for a graduate fellowship. Nonetheless, she was turned down by one university after another. She could not pay for a high education and most importantly, PhD graduate programs were not eager to provide assistant positions to women in physics. The admissions office believed that, as a woman, much less a Jewish woman, she would never get a job in the field.

Yalow was well prepared for these setbacks. While still in college, she had taken typing courses so she could support herself as a secretary. In 1941 she obtained a part time position as a secretary for a biochemist at Columbia University in exchange for graduate classes, but she had to agree to take stenography at a business school. In her biography Yalow wrote: “Fortunately I did not stay there too long. In February I received an offer of a teaching assistantship in physics at the University of Illinois, the most



*Rosalyn Yalow, 1977 [24]*

prestigious of the schools to which I had applied. It was an achievement beyond belief. I tore up my stenography books and during the summer took two tuition-free physics courses at New York University” [4].

Twenty-year-old Rosalyn arrived at the University of Illinois in 1941 and was not only the first woman graduate student in the physics department at Illinois since 1917, but again the only woman among 400 members of the College of Engineering faculty [2, 3].

On the first day of graduate school Rosalyn met Aaron Yalow, who was the son of an Orthodox rabbi, and was also beginning graduate study in physics at Illinois. Friendship between them turned into love but according to the rules of the University, they could not enter into marriage as long as they were both teaching assistants in the department. Rosalyn made a decision about both marriage and career and never doubted her ability to achieve those two goals. They married in 1943 when Aaron got a fellowship elsewhere [7].

The years at the University of Illinois were a busy time. Rosalyn successfully dealt with graduate courses, a heavy assistant teaching load, long hours in the laboratory and war-time housekeeping. Her academic performance was outstanding. She received 21 A's in her courses and one A- in the electrodynamics laboratory. In her words, “The Chairman of the Physics Department, looking at this record, could only say that A-confirms that women do not do well at laboratory work. But I was no longer a

stubborn, determined child, but rather a stubborn, determined graduate student. The hard work and discrimination were of no moment”[4]. The fact that she was a self-made woman, who had to rely on personal intelligence and determination to get ahead; was a point of pride for Yalow.

During research work she became skilled at making and using equipment to measure radioactive substances. Rosalyn’s doctoral thesis was on “Doubly ionized K-shell following radioactive decay.” In 1945 the Yalows received their Ph.D. in nuclear physics, returned to the Bronx and both found jobs at the Federal Telecommunications Laboratory. Rosalyn worked there as assistant engineer – again the only woman engineer [8].

Rosalyn wanted a research position in medical physics, but such jobs were not routinely offered to women. So she returned to Hunter College, her alma mater, and accepted a full-time position in teaching physics.

Throughout her career, Rosalyn advocated for including more women in science. While attending Hunter, she took interest in excelled student Mildred Dresselhaus. Yalow encouraged young Mildred to apply for graduate fellowships and was responsible for leading her away from primary school teaching into a research career in physics [9]. In the future, Mildred became the first to predict band structures of carbon nanotubes and became famous in the scientific world as the “Queen of Carbon Science” [10].

Rosalyn was intensely interested in medical research and in 1947 through her husband’s help, began her long association with the Bronx Veterans Administration (VA) Hospital. It was a real turning point in Yalow’s career that ultimately led to the development of radioimmunoassay.

She joined the Bronx VA Hospital at the age of 26 as a nuclear physics part time consultant with a small salary. This Hospital was involved into a research program to explore the use of radioactive substances in medicine. After the war, nuclear reactors allowed to produce short-lived inexpensive radioisotopes for research and its application in medical treatment seemed very promising. Yalow was aware that this was a new area in which research had to proceed along with clinical application. Her task was to develop the Radioisotope Service.

Her engineering skills proved helpful and she could design and make much of the needed equipment that was not available on the commercial market. By 1950, 29-year-old Rosalyn established



*Rosalyn Yalow in the laboratory. She conducted her research at the VA Medical Center in Bronx, New York, from 1947 to 1991 [3]*

a radioisotope laboratory in the Bronx VA, which was one of the first in the United States. She was appointed assistant chief of the radioisotope service, left her teaching position for full-time research and started projects in a number of the clinical field with physicians in the hospital. In her words, “Though we started with nothing more than a janitor’s closet and a small grant from a veterans’ group, eight publications in different areas of clinical investigation resulted from this early work” [4].

Yalow’s scientific biography can be viewed as a summary of her hard self-sacrificing work, her good luck, and her bad luck. The good luck was her opportunity to work with Sol Berson, her bad luck was having him die prematurely at age 54 in 1972 [11].

Rosalind Yalow was an ordinary physicist working in a new field of physics and did not have enough competence in medicine. Moreover the ideas of radioisotopes application in medicine were far from the scientific mainstream at that time and have not yet been accepted in the clinical field. So Yalow began looking for a collaborator to complement her competencies and not to miss her chance. In the spring of 1950, she met Solomon Berson, a highly recommended young medical doctor who had just completed his internship at the VA Hospital. Like Yalow, he had faced academic anti-Semitism and was rejected by dozens of medical schools. He was a

multi-talented scientist and had a vast knowledge in biology, physiology, anatomy and clinical medicine. Berson joined Rosalyn's small team and, within four years, became the chief of the radioisotope unit. Yalow and Berson complemented each other in many ways, their research partnership lasted for 22 years; people who knew how they worked reported that they sometimes finished each other's sentences and even joked about believing in telepathy [1, 12].

One of their first joint investigations were in the application of radioisotopes to rapid determine of thyroid activity. Because gamma-radioactive Iodine-131 or Iodine-125 atoms can be easily attached to tyrosine residue of proteins they injected radioisotopically labeled substances into the blood, took blood samples after the injection and in a 35-minute session using mathematical analyse determined the rate of iodine removal from the blood stream by the thyroid gland [13].

These studies became the basis for investigating the metabolism of  $^{131}\text{I}$ -labeled insulin. They turned to insulin for several reasons. Insulin was the hormone available in a highly purified form and easy to work with in the laboratory. Among endocrine diseases, diabetes affects the largest number of people who have to take injections of exogenous insulin obtained from the cattle pancreas. Besides, Yalow was especially interested in insulin as her husband Aaron was a diabetic. Yalow and Berson wanted to learn what happened to insulin once it entered the bloodstream.

To begin, Yalow and Berson injected intravenously the minute amounts of  $^{131}\text{I}$ -labelled cattle insulin into non-diabetic volunteers, including themselves and diabetic patients, and measured how fast the hormone disappeared from the blood samples taken in time points of several hours after injection. They were surprised to observe that radioactive insulin disappeared more slowly from the plasma of patients who had received insulin, either for the treatment of diabetes or as shock therapy for schizophrenia, than from the plasma of subjects never treated with insulin. What was the cause of the observed effect? The electrophoresis patterns of labeled insulin in the plasma showed that in insulin-treated patients the labeled insulin was bound to and migrated with an inter beta-gamma globulin. So, they concluded that the retarded rate of insulin disappearance was due to the binding of labeled insulin to antibodies that had developed in response to the administration of exogenous insulin.



*Rosalyn Yalow with her research partner Solomon Berson, when she won the 1961 Ely Lilly Award of the American Diabetes Association [3]*

In the mid 1950's this concept was not acceptable since the immunologists were traditional in their ideas that insulin molecule were too small to provoke antibody production and beside the analysis of reactions between antigens and antibodies was restricted to those that produced visible precipitation or the clumping of red blood cells. According to Yalow, "these classic immunologic techniques were not adequate for the detection of antibodies to insulin which were likely to be of such low concentration as to be nonprecipitating" [14].

The findings of Yalow and Berson were the first real evidence that such a small protein as insulin could stimulate an immune response. Based on these data, the two scientists concluded that diabetics should not use bovine insulin but human insulin, which will not be susceptible to antibody attacks. Today, genetically modified bacteria are used to produce large amounts of insulin precisely the same as humans.

The first attempt to publish an original paper describing Yalow and Berson's findings was rejected by *Science* and initially rejected by the *Journal of Clinical Investigation*. In her Nobel lecture, Yalow presented a copy of the rejection letter, dated September 29, 1955, stating that "...the experts in the field believe that you have not demonstrated an antigen-antibody reaction, nor that you have proved that

a globulin is responsible for insulin binding, nor that insulin is an antigen". A compromise with the editors eventually resulted in acceptance of the paper, but only after "insulin antibody" was omitted from the title and the conclusion that the binding globulin was indeed an antibody was documented by showing how it met the definition of antibody given in a standard textbook of bacteriology and immunity [14].

In this paper the authors also reported that the binding of labeled insulin to a fixed concentration of antibody is a quantitative function of the amount of insulin present in the blood samples [15].

Thus Yalow and Berson not only demonstrated for the first time the production of antibodies against insulin, but also developed the breakthrough radioisotopic technique to measure the endogenous insulin concentration. Investigations on the quantitative aspects of the reaction between insulin and antibodies lasted for several years [16, 17] and finally led in 1959 to the measurement of insulin in unextracted human plasma [18].

Yalow and Berson called their method radioimmunoassay (RIA). Thus, it can be said that the era of RIA began 1959. The RIA test is simple in principle and is based on competing reactions. Three primary substances are present in the reaction: radiolabelled antigen ( $I^{125}$ -insulin), unlabelled antigen (insulin from blood samples or standards), and specific antibody to insulin (Fig.). The radiolabelled antigen ( $I^{125}$ -insulin) is mixed with a known amount of its antibody, resulting in the formation of a labelled antigen-antibody complex (1). Then, a quantity of the patient's blood, in which there is an unknown quantity of the naturally occurring insulin as unlabelled antigen, is added (2). The new unlabeled antigen competes for the antibody binding sites with the radiolabelled antigen and replaces it (3). The more insulin in the blood sample, the more labeled insulin is displaced from the complex and released. The antigen-antibody complexes formed are separated by precipitation (4), the radioactivity of both the radiolabeled bound and radiolabeled free antigen is measured using a gamma counter, and their ratio is calculated.

With data obtained in a series of standard samples in which the concentration of antibody and the radiolabelled antigen is kept constant, while the concentration of unlabelled antigen is varied, a binding standard dose-response curve is constructed from which the unknown concentration of hormone can be read. As little as a femtomole of hormone, i.e.

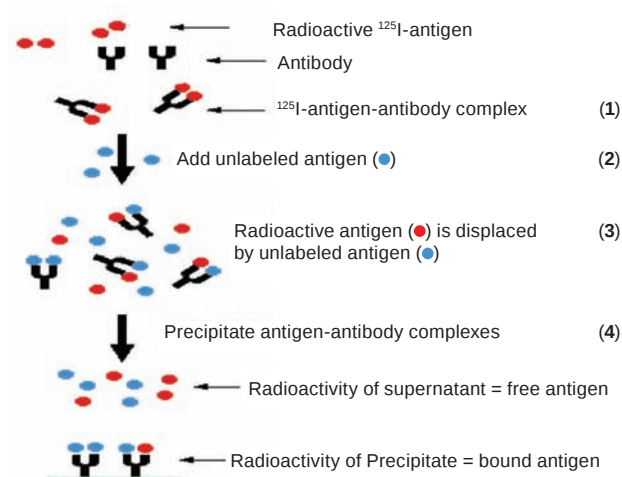


Fig. The diagram of how RIA works (modified) [25]

the amount present in 1 ml of a  $10^{-12}$  M solution, can be detected.

This technique, for the first time, allowed us to observe the previously invisible antigen-antibody reactions in solution and to detect hormone levels in the blood by an *in vitro* assay. In addition to its remarkable sensitivity RIA was a test-tube operation with no radioactivity entering a patient's body! This was revolutionary in theoretical immunology, all biology, and in applied medicine. It ensured a higher quality of life for diabetics, as they could now monitor the concentration of insulin in their blood and correct it with injections as necessary.

Now the RIA technique is automated and computerized. But at the time, Berson and Yalow worked for days preparing solutions in thousands of test tubes, running tests day and night, with no technicians to help, without a single research grant, and doing all the work themselves. The technique was so new and complicated that they spent the following years developing the RIA concept into practical use. They also began training scientists to use RIA.

Over the next decade, their group developed RIAs for growth hormone, adrenocorticotrophic hormone, parathyroid hormone, and gastrin. In each case, these new assays led to important new insights into the role of these hormones in normal physiology and how the role was altered in disease states [19].

Moreover, RIA could be used for a variety of other biologically important agents. In Yalow's Nobel Lecture, she listed more than 100 biological substances – hormones, drugs, vitamins, enzymes, viruses, non-hormonal proteins, and more – that one could measure using RIA.

By the end of the 1960s the significance of their discovery was recognized, and the RIA field exploded, this technique began to be widely used in laboratories around the world and soon found hundreds of applications, in particular to screen blood for hepatitis virus in blood banks, determine effective dosage of drugs, treat dwarfed children with growth hormones, and correct hormone levels in infertile couples, for the early detection of cancer, and to measure the levels of neurotransmitters.

Although the commercial potential for their new technique was tremendous, Yalow and Berson decided, like the Curies, not to patent their discovery. If Rosalyn Yalow had patented RIA, she would be a rich woman. But she never regretted not doing so and claimed that she always felt uncomfortable having more money than she could spend usefully. According to Yalow, "We never thought of patenting RIA. Of course, others suggested this to us, but patents are about keeping things away from people for the purpose of making money. We wanted others to be able to use RIA" [20].

The practical importance of the RIA concept cannot be overestimated. Due to virtually unlimited applicability, it has become the basis for variety of other immunoassays techniques with the use of the linked enzyme or fluorescent marker in place of radioactive label. For example a widely used today enzyme-linked immunoabsorbent assay (ELISA) involves labelling of the antibody with the enzyme, usually a horseradish peroxidase or alkaline phosphatase able to catalyze a colorimetric reaction when exposed to the appropriate substrate, while the unlabelled antigen is attached to the wells of the microplate to facilitate separation of the antibody-ligand complex and washing. While ELISA is safer, faster, less costly for scientists and is better for testing large numbers of samples quickly, RIA assay is more sensitive and specific and is better for accurate detection and quantification of low levels of target molecules in complex biological samples [21].

Throughout the 1970s Yalow's work was highly recognised at VA Hospital. She became the director of the Radioimmunoassay Reference Laboratory and the head of the Nuclear Medicine Service. Berson's unexpected death of a heart attack in 1972 was Yalow's low point, both professionally and personally. At her request the laboratory which they shared was renamed the Salomon A. Berson Research Laboratory in honor of her collaborator of 22 years. After his demise, Yalow had to show that she was "more than just his technician".



*Rosalyn Yalow in the laboratory [3]*

Yalow continued to work and published 60 articles between 1972 and 1976 about parathyroid and gastrointestinal hormones metabolism. She was elected to the National Academy of Sciences in 1975, and in the next year became the first woman to be awarded the Albert Lasker Prize for Basic Medical Research. Although extremely prestigious in its own right, the Lasker Prize is generally considered a precursor to the Nobel Prize in Physiology or Medicine. And, as expected, Yalow received this award in 1977. She was awarded half of the Nobel Prize for Physiology or Medicine, "for the development of radioimmunoassays of peptide hormones." Her only regret was that Berson was not alive to share the triumph with her. The other half was shared jointly by Roger Guillemin and Andrew Schalley, who extensively employed RIA to discover and measure a number of hormones that are released by the brain [22].

Yalow was the second American woman to receive this honor. The first was Gerty T. Cori in 1947, who shared it with her husband "for their discovery of the course of the catalytic conversion of glycogen", but Yalow was the first woman who was born and educated in America to win the Nobel Prize in a scientific field.

After the excitement and publicity of the Nobel Prize, the tireless Yalow continued to conduct and direct research at her laboratory in VA Hospital until her retirement in 1991 at the mandatory age of 70. Over the course of the 1990s, Yalow suffered from a series of strokes and with her sudden stroke on January 1, 1995, was taken to a hospital. In 1997, while rehabilitating, she was able to return to her lab.

She continued to respond to mail from students and give brief interviews until she died on May 30, 2011, in the Bronx, New York at the age of 89.

Rosalyn Yalow was undoubtedly an outstanding and extraordinary personality. She was a staunch individualist in her dealings with other female scientists and has been called self-sufficient and highbrow. She felt that her ambition made her female peers dislike her and, throughout her life, she had very few female friends who weren't scientists [1].

The biography of this somewhat aggressive, extremely competitive and complex woman illustrates the price women must pay to pursue a career in science. According to her all women scientists should marry, rear children, cook, and clean to achieve fulfillment, and be a complete woman. While working at the VA, Rosalyn and Aaron had two children a son Benjamin and a daughter Eline. According to policy at the VA, women had to leave once they were five months pregnant, but both times Yalow ignored it. Moreover, a week after giving birth, the young mother returned to her laboratory together with her child. With the help of her mother, and an understanding husband who supported his ambitious partner, she combined career, motherhood and maintaining a home. She was home in time to prepare dinner every evening, sometimes returning to her laboratory afterward, working long into the night. In an interview many years later, she said, "It's true that women are different from men. If you want to be a good wife, you have to work a little harder" [23]. She routinely maintained a work schedule of sixty to eighty hours per week.

While she encouraged and supported young women with promising scientific potential, she neither supported women's organisations nor admitted the existence of gender issues. She was against the idea that women should be treated any differently from men in the workplace and refused to accept awards restricted to women, which she regards as a sign of reverse discrimination [6].

A few years after Yalow died, in 2015, a charter school opened in the Bronx where she grew up and was named in her honor [5]. The school wrote in its mission statement, "Our hope is that Dr. Yalow's legacy will inspire a new generation of Bronx children to work hard at their education and strive for success."

Yalow's name, like that of many other female scientists, has been partly forgotten. However, an invaluable contribution to the development of radioim-

munoassay, which influenced all aspects of medicine and biology, as well as the triumphant overcoming of gender and religious barriers in the field of experimental research distinguish this women as one of the extraordinary figures of the last century and ensure her a honourable place in the history of scientific development.

### **РАДІОІМУНОЛОГІЧНИЙ АНАЛІЗ І РЕВОЛЮЦІЯ В МЕДИЧНИХ ДОСЛІДЖЕННЯХ: ЛАУРЕАТ НОБЕЛІВСЬКОЇ ПРЕМІЇ З ФІЗІОЛОГІЇ ТА МЕДИЦИНИ (1977) РОЗАЛІН ЯЛОУ – ВЧЕНИЙ ІЗ БОЙОВИМ ДУХОМ**

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Розумна, але бідна нью-йоркська дівчина, яку батьки бачили в майбутньому лише як вчительку, Розалін Ялоу після закінчення школи в 1937 році вступила до виключно жіночого коледжу Хантер і була першою жінкою з дипломом фізика, коли бути жінкою, а тим більше єврейкою, було серйозною перешкодою для успіху. Незважаючи на те, що її називали дещо агресивною та високолобою, вона стала першою жінкою-студенткою на фізичному факультеті Університету Іллінойсу, єдиною жінкою серед 400 членів інженерного факультету Коледжу, а після отримання докторського ступеня – єдиною жінкою-інженером в Телекомунікаційній лабораторії Бронкса. До 1950 року вона заснувала радіоізотопну лабораторію в лікарні ветеранів Бронкса, одну з перших у Сполучених Штатах. У співпраці з доктором медичних наук Соломоном Берсоном, дослідницьке партнерство з яким тривало 22 роки, Ялоу досліджувала метаболізм міченого  $^{131}\text{I}$ -інсуліну у хворих на цукровий діабет. Розалін Ялоу не лише вперше продемонструвала продукування антитіл проти такого маленького протеїну, але й вперше розробила радіоімунологічний аналіз (RIA) для вимірювання концентрації інсуліну в крові пацієнта без потрапляння радіоактивності в організм. Цей революційний метод почав широко використовуватися в усьому світі для вимірювання різноманітних біологічних ре-

човин. У 1977 р. Розалін Ялоу була удостоєна Нобелівської премії з фізіології та медицини «за розробку радіоімунних аналізів пептидних гормонів». В огляді розповідається про наукову кар'єру та життєвий шлях цієї незвичайної жінки.

**Ключові слова:** Розалін Ялоу, радіоімуннологічний аналіз, інсулін, Нобелівська премія, ЕЛІЗА.

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