

NICOLAE CONSTANTIN PAULESCU: THE FIRST EXPLICIT DESCRIPTION OF THE INTERNAL SECRETION OF THE PANCREAS

NICOLAE CONSTANTIN PAULESCU: PRVI EKSPLICITNI OPIS UNUTARNJEG IZLUČIVANJA GUŠTERAČE

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SUMMARY

The purpose of this article is to describe the research of Nicolae Constantin Paulescu and to emphasize his role in the discovery of insulin. Methods: We made a thorough review of the literature and research in the Romanian Academy Archive in order to find adequate references. Results: In 1912 N.C. Paulescu analysed the clinical and biochemical alterations in diabetic patients and in dogs after performing a pancreatectomy, that apart hyperglycemia and glycosuria (carbohydrate metabolism), had noted also changes in lipid and protein metabolism. In 1916 he started the experiments with a pancreas extract obtained by his original method, that was injected intravenously to the diabetic dogs. The results of his first experiments showed: "The pancreatic extract injected into a peripheral vein produce: 1) A diminution and even a temporary suppression of diabetic hyperglycemia, which may be replaced by hypoglycemia; 2) A diminution or even temporary suppression of glycosuria; 3) A diminution of blood urea; 4) A diminution of urinary urea. In other words, the intravenous injection of the pancreatic extract has as effect the disappearance of diabetic symptoms. The attenuation of the diabetic syndrome begins immediately after the injection. It reaches a maximum

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after 2 hours,- and it lasts for about 12 hours". He concluded as such: "This discovery,- which sheds a bright light over the pathogenesis of diabetes gives us also the key for the treatment of this syndrome". In 1921, Paulescu had published extensively his data in two outstanding French journals 8 months before the first publication of Banting and Best from February 1922. It is clear that insulin has been discovered in Europe. Conclusion: Paulescu thought that a new hormone – Pancreine, that he discovered is the key element in the treatment of diabetes, but his outstanding research was unfairly neglected.

Keywords: insulin discovery; medical ethics; chronology and context; experimental design.

INTRODUCTION

The discovery of insulin has been considered the greatest discovery of the last century and one of the greatest of medicine in modern age [1-3]. However, Michael Bliss, in his monograph, "*Discovery of insulin*", [1] in chapter 8, entitled "*Who discovered insulin?*", couldn't give a clear response to this question. It is obvious that the clinical application of the newly discovered hormone made in 1922 in Toronto, raised an explicable enthusiasm among the medical community of that time. Now we know that the purification of the pancreatic extract, which makes possible its utilization in human, has been done by Collip in January 1922 [4] and that its extract was the only one used in the first clinical trial carried out in Toronto before the industrial production put in work by Lilly Company in the second part of 1922 and then afterward [4].

In 1923, the Nobel Prize was awarded to Frederick Banting and James Macleod, but the official Canadian historiography of that time had a preference to Banting and Best (the first, an untrained country doctor, and the second, an assistant student). Why? Because the big discovery was transformed by Canadian mass-media and political circles in a fairy story about what was called the "native Canadian genius erupting into a miraculous achievement". In fact, out of four members of the famous "Toronto's crab basket" (Banting, Macleod, Best and Collip), only the first two received an international recognition and glory [1]. It is not the place to analyse on what "objective" basis was awarded the Nobel Prize in 1923, but a letter of Prof. Alfred Pettersson (a member of the Nobel Prize jury) to the Nobel Assembly (mentioned by Michael Bliss in his book, page 228), is illustrative:

"It is quite clear to me that a fundamental requirement in awarding a person a Nobel Prize is knowledge of what part the person has actually taken in the work being honoured. During the time I have participated in the awarding of the Nobel Prize, the justification for the award has never been based on hearsay evidence from unknown persons, on statements like "it is beyond doubt",

*on things that are thought as “very possible”. In my opinion, it is very necessary that **the Assembly risks the development of unpleasant discoveries at a later date.** I also point out a certain contradiction in Professor Jacobaeus’s final judgement about Macleod’s part in the work relating to insulin production. Banting is said to have been ready to make an experiment that would not have led to the goal, and to have been corrected by Macleod. But before that, Jacobaeus writes that Banting came with his idea to Macleod and worked through to insulin under Macleod’s direction. If the work was totally under Macleod’s direction, then Banting could hardly be made responsible, at least not alone, if they, in the beginning, started out on the wrong road.”*

It is interesting to remember that the members of the Committee had big difficulties in the identification of what achievements Banting and Macleod had made, because their dossier was very “thin”, with few and inconclusive published papers [5,6]. Finally, the Nobel Prize has been awarded to the “humble Canadian genius” – Frederick Banting, and to the refined Professor James Macleod.

The prophecy of Alfred Pettersson has been fully accomplished. It is well known that 1923 Nobel Prize awarding for Medicine was the subject of many critics and disputes, which hopefully will end in 2021, with the occasion of the celebration 100 years since the insulin discovery.

THE ROAD OF DIABETES UNDERSTANDING BEFORE INSULIN DISCOVERY

The modern history of diabetes included a chain of events starting with the rediscovery of the sweet taste of urine by Thomas Willis (1621-1675) and by the observation of Eugène Chevreul (1786-1889) that sugar from the urine of diabetic patients is identical with the sugar from grapes. Claude Bernard (1813-1878) introduced the term “internal secretion” and discovered the glyco-genic function of the liver, which maintains blood glucose around 120 mg/dl, and that the renal threshold for glycosuria is blood glucose between 170-180 mg/dl. Paul Langerhans (1847-1888) described some specific agglomeration of small cells that can be seen from place to place among the acinary histologic appearance of the pancreas. Etienne Lancereaux (1828-1910) described for the first time the pancreas as the organ affected in diabetes, introducing the term *pancreatic diabetes* (which is specific for “thin” diabetes – the actual type 1 diabetes); in the same time, he described also the “fat” diabetes (the actual type 2 diabetes), producing the first classification of the diabetes syndrome.

The pancreatic origin of diabetes has been incidentally confirmed by von Mering and Minkowski in 1889. Edouard Lague sse (1861-1927) rediscovered the doctoral thesis of Langerhans with the histologically description of pancreas and suggested that these “islets of Langerhans” could be the siege of the internal secretion of the pancreas. Emmanuel H don (1863-1933) provided indirect evidence of an ‘anti-diabetic principle”, produced by the normal pancreas. Between 1893-1919, several dozen of researchers tried to prepare various pancreatic extracts in order to treat diabetic patients and eventually, to understand the function of the supposed pancreatic anti-diabetic hormone. The presence of this was quite evident, explaining why in 1909, the Belgian physiologist Jean de Meyer [7], proposed that when this hormone will be discovered to be named “*Insulin*”.

It is well to mention that in all these attempts diabetes has been interpreted as a “sugar disease”, so that the only investigation used for proving the presence of a pancreatic internal secretion was based on the determination of urinary glucose and sometimes of blood glucose.

PAULESCU AND THE NEW PARADIGM OF DIABETES

The interest of Paulescu (1869-1931) for the internal secretion of the pancreas dated back to 1899, when, together with Albert Dastre (1844-1917) at the Sorbonne University, he tried to obtain a pancreatic extract to be used for the treatment of diabetes. This project was postponed because in 1901 Paulescu returned from Paris to Bucharest, in order to create the first Physiology Chair in the young Faculty of Medicine. He maybe understood that before obtaining a therapeutic “tool”, it was necessary to know the pathophysiologic basis of diabetes. In other terms, to know the nature of the internal secretion of the pancreas and its physiological properties. This enterprise lasted at least 14 years, including the 4-year break imposed by the 1st World War.

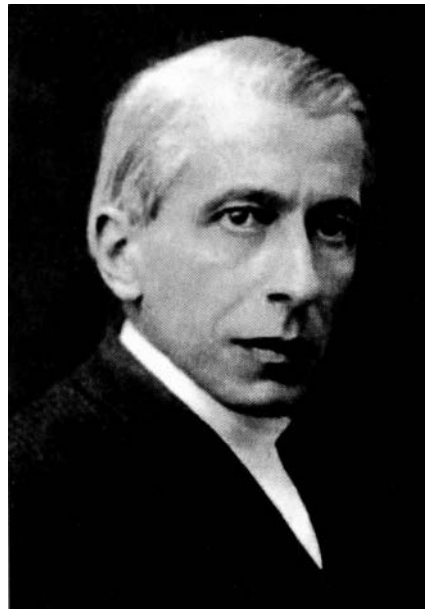


Figure 1. N.C. Paulescu in 1907.

The most comprehensive papers published by Paulescu in which were described all physiologic functions of the new hormone isolated by him (named "Pancreine") and also its pharmaco-dynamic characteristics, were published in two outstanding journals: the first on 23 July 1921 as four short papers [8-11], and the second one, on 31 August 1921 in Archives of Internal Physiology Liège/Paris, with an unexpected title "*Research regarding the role of the pancreas in nutrient assimilation*" [12]. We will refer to this paper in detail later.

Here we have to mention that, for the first time, diabetes has been presented not as a disorder of carbohydrate metabolism, but as a disorder of an entire energy metabolism of the human body, including also lipid and protein metabolism. In fact, such interpretation made an important shift in diabetes paradigm, whose importance was understood as such only in the last decade when the researchers became aware that diabetes was not only "mellitus", but also "proteinus" [13] and "lipidus"[14].

When this new concept of diabetes has been born? It is difficult to say. The first written document from Paulescu's papers dated back from 1907, when in the "*Handbook of Physiology*" (990 pages in Romanian) we found the following sentence [26]:

"The pancreas plays an important role as a gland with external secretion; apart this function, it also plays an important role as a gland with internal secretion, function discovered by Lancereaux in 1877, in diabetic patients, when he found their pancreas to be altered.

*In the pancreatic diabetes the man had either progressively or suddenly, an extraordinary thirst, which cannot be relieved. The urine contains a large quantity of glucose; also, the quantity of the eliminated urea is increased up to 150 g/day. Concomitant with that, the patient had intense hunger, and despite a high food intake, he continually lost weight, **a prove that assimilation cannot be made (or underlined)**. And then:*

"The total ablation of the pancreas induces the same type of diabetes as that of humans: glucose, urea, hunger, thirst and death".

In a subchapter, "*The cause of pancreatic diabetes*", Paulescu wrote:

"Ligating Wirsung duct in dogs, diabetes did not appear; so, the pancreatic diabetes is not due to non-penetration of pancreatic juice in the intestine. By removing the head of the pancreas and its duct, we don't obtain diabetes. Moreover, if we introduce a piece of pancreas under the skin, and after a while we remove the pancreas, we also cannot obtain diabetes."

In the last paragraph of this chapter, he concluded:

“So, the pancreas is a gland with internal and external secretion. The internal one plays a role in preparing glucose for its assimilation. When the pancreas is removed, the liver cannot store glucose as glycogen.” [16,17]

In order to more precisely place the moment when Paulescu arrived at a conviction of the fundamental role of the pancreas in glycogen homeostasis, we need to refer to a series of experiments effected by him between 1903-1911, when he perfected the original surgical technique of total pancreatic ablation, associated sometimes with the extirpation of a hepatic lobe in order to study the effect of pancreatectomy on hepatic glycogen in diabetic dogs as well as in “fluorizinic” diabetes [17-23].

“Following total pancreatic ablation, the power of the liver to store glycogen is considerably reduced”. “Less affected is muscular glycogen and totally unaffected is myocardial glycogen”. “In pancreatectomised dogs, the capacity of the tissues to store glycogen is not totally lost only diminished. This is a secondary phenomenon - that is, a consequence not a cause of diabetes.”

On this occasion Paulescu discovered the “incretine effect” following the observation that the intraportal administration of glucose has any effect on the glycogen accumulation in the liver, in contrast with the same amount of glucose, administered orally induces a rapid accumulation of glycogen into the liver [23,24].

A more comprehensive description of diabetes appeared in the 3rd volume of the *“Traité de Médecine Lancereaux –Paulesco”, Paris 1912* [25]. From the chapter entitled *“The Pancreas”*, and the subchapter ‘Physiology’ (pages 925-927) the following text is reproduced:

“II. Apart from the role played by the pancreas in processing food for their absorption, it carries out an equally important function through which it participates in the preparation of these absorbable substances, especially of carbohydrates, for their utilisation.

Lancereaux was the one who, in 1877, brought this hitherto only suspected function to light by describing a form of diabetes whose pancreases were affected by a morbid process. He felt that this form of diabetes was a consequence of functional suppression of the pancreas and he named it PANCREATIC DIABETES (E. Lancereaux. Bull. Acad. Médecine, 1877, page 1215)

Only twelve years later in 1899, two physiologists (von Mering and Minkowski), carried out total ablation of the pancreas in dogs obtaining in these animals, a form of diabetes analogous to that in man and confirmed, in an experimental manner, Lancereaux's earlier clinical conclusions.

We will return to pancreatic diabetes in humans later (page 933). For now, we shall report several experiments which help to elucidate the pathogenesis of this important syndrome permitting us to understand it.

Total or subtotal pancreatic ablation leads to the appearance of severe diabetes, which consists of considerable hyperglycaemia, azoturia (urine urea nitrogen) and polyphagia. Although they eat a lot, the operated animals lose weight rapidly and die in a state of cachexia. Very frequently, the healing of the operation sites is very slow or does not occur at all.

Partial ablation of the pancreas does not lead to diabetes, if the part remaining does not have less than one-tenth of the weight of the organ. If the fragment remaining does not reach this dimension, a more or less profuse post-prandial glycosuria results, that is, the appearance of glucose in the urine, especially after a meal rich in carbohydrates.

Ligation and resection of the excretory ducts of the pancreas are not followed by diabetes. The same happens after extirpation of the entire duodenal portion of the organ if the portion remaining in the abdomen is sufficiently voluminous - even though it is lacking in the excretory canals which drain its secretions into the duodenum. Thus, diabetes is not a consequence of a defect in the flow of pancreatic juice into the duodenum; in other words, it is not a consequence of the suppression of the pancreas' exocrine secretion.

These experimental facts also prove that the lesions of the abdominal nervous plexuses occasioned by the extirpation of the pancreas play no role in the onset of diabetes as some authors claim.

Now, the question is: how does an endocrine suppression of the pancreas cause diabetes? Many hypotheses have been propounded to answer this question, but not one of them has a solid and indisputable basis.

Grafting of the pancreas and the fact that the liver and muscles of depancreatized animals no longer contain glycogen have made us admit that until we have new data, the products of the internal secretion of the pancreas act on sugar - which is carried to the liver through the blood of the portal vein - and leads to some modifications which permit it to be first metabolised - that is, to be stored as glycogen by the liver, muscles etc. - and later to be utilised

(consumed) by the tissues. In the absence of the endocrine secretion of the pancreas, the sugar from the blood, being unassailable, is no longer fixed (in the liver) in the form of glycogen, nor utilised by the tissues. It accumulates in the blood (hyperglycaemia) producing osmotic effects (dehydration of the tissues, polydypsia) and being unusable (weakness, azoturia (urine urea nitrogen) and polyphagia), is eliminated in the urine (glycosuria) as a foreign body." A wonderful synthesis of the pathophysiology of diabetes, which can be reproduced in any handbook of diabetes even today.

THE EXPERIMENTAL DEMONSTRATION OF THE NEW PARADIGM

According to our knowledge today, diabetes expresses a complex disorder of the energy metabolism, i.e. a defect in the peripheral utilization of all energy fuels: carbohydrates, lipids and proteins. This definition confirms the 100-year old vision of Nicolae Paulescu, the man who changed the paradigm of diabetes, considered until then a disorder affecting only the carbohydrate metabolism, into a disorder in the utilization of all energy fuels.

In order to understand Paulescu's manner of thinking and acting, it must be said that his first source of information was the careful analysis of diabetic patients. Like a detective who has some indirect clues related to the guilty and gathers meticulously the evidence leading to the right conclusion, Paulescu used clinical and biochemical observations of human diabetes, but also the symptoms appeared after total pancreatectomy in dogs. He observed that the symptoms of diabetes can be explained by an accelerated catabolism of proteins (weight loss) and the accumulation in the circulation of biochemical compounds, such as glucose and ketone bodies demonstrating the defect in their utilization in peripheral tissues. The second information comes from the symptoms appearing after total ablation of the pancreas: the sudden onset of the same symptoms. Paulescu noted that the use of all fuels in the tissues is affected.

In order to demonstrate that this defect is due to the absence of the antidiabetic pancreatic hormone, he assumed that its intravenous administration should lead to the correction of all three changes (in glucose, lipids and proteins) in blood and urine considered by him to be the "*cardinal symptoms of diabetes*".

From his published papers we can reconstitute the experiments he used: total pancreatic ablation of healthy dogs, in order to induce experimental diabetes; extraction from the pancreas of "the antidiabetic substance"

(unknown at that time) using an original method, giving maximal attention to aseptic measures and the respecting the conditions that would preserve the active principle (temperature below 50° C). A such extract to demonstrate its effects on blood and urinary glucose, ketone bodies and urea.

In 1916, towards the end of his experiments in which this hypothesis was validated, his research was interrupted by the 1st World War. Laboratories were closed and the publication of his work done till then in an international journal was impossible. This is the reason why the data obtained by him before the War (with the exception of the effects on ketone bodies) appeared in an exhaustive form in the 2nd volume of “*Traité de Physiologie Médicale*”, published in 1920 in French [26].

At the end of this chapter, Paulescu made very clear the following statement: “*This discovery, which sheds a new light on the pathogenesis of diabetes, also gives us the key to the treatment of this syndrome.*”

After the reopening of laboratories in 1921, Paulescu demonstrated the last effect of Pancreine on ketone bodies, also experimentally proving the specificity of the effects, that were not noted after the administration other organs' extracts, or by inducing a bout of fever with pyrogenic substances. These data were published in 4 short papers on 27 July 1921 in the outstanding publication of the Biology Society in Paris (Compte Rendu de la Société de Biologie du Paris) [8-11], and finally, in a more comprehensive manner, in International Archives of Physiology (Archives International de Physiologie) (Liège, Paris), on 31 August 1921 [12]. The conclusions of this famous paper which can be considered the “**true insulin's birth certificate**” were:

I. “*If in an animal, with diabetes induced by ablation of the pancreas, a pancreatic extract is injected into the jugular vein, we observe:*

- a). *Diminution and temporary suppression of hyperglycaemia, which may be replaced by hypoglycaemia and a diminution or even temporary suppression of glycosuria;*
- b). *Considerable diminution of blood urea and urinary urea;*
- c). *Marked diminution of ketonaemia and ketonuria.*

II. *The effect of the pancreatic extract on glycaemia and glycosuria varies with the interval of time following the injection, beginning immediately after the injection and reaches a peak at about 2 hours and lasts about 12 hours.*

The effect also varies with the amount of pancreas used for preparing the extract.

III. *If in a normal non-diabetic animal a pancreatic extract is injected into a vein, a marked diminution of glycaemia, blood urea and urinary urea is noted.*

IV. *Similar effects, influencing especially diabetic hyperglycaemia and glycosuria are not produced by either:*

a.i.v. injection of a saline solution;

b.i.v. injection of an extract of an organ other than the pancreas;

c. Or intraspinal injection of a sodium nucleate solution causing a bout of fever.”

We do not believe that a single line of this “Certificate” can be contested. All the metabolic functions of the newborn hormone were clearly described in such a manner that they remain as we know even today. As happened with many visionary concepts, this view was over sides by Paulescu’s contemporary. This paper had been published 8 months before the first experimental work of Banting and Best in February 1922, and was known by at least three of the Canadian team (Macleod, Banting and Best), according to the written documents from the Bliss book [1].

HOW AWARE OF PAULESCU’S WORK WAS THE CANADIAN TEAM?

In his book [1], Michael Bliss tried to convince us that Paulescu’s work was not known by North-American researchers, including the Canadian team. Nothing could be more wrong. As early in November 1921, Ernest Lyman Scott (1877-1966) (who produced a pancreatic extract years earlier) sent to Paulescu a congratulatory letter for his paper published in 1921. John Raymond Murlin, another known researcher in the field, stated in 1923, that he resumed his research on the pancreatic extract stimulated by Paulescu’s papers. In their first paper, published in February 1922 Banting and Best, quoted Paulescu’s papers [5,6,8] in a strange manner:

“Paulescu has recently demonstrated the reducing effect of whole gland extract upon the amounts of sugar, urea and acetone bodies in the blood and urine of diabetic animals. He states that injections into peripheral veins produce no effect and his experiments show that second injections do not produce such marked effects as the first.”

This obvious misrepresentation of Paulescu's very clear data has not been explained till today. It has been suggested that the ignorance of "young researcher" stayed at the base of this ignoble misrepresentation. If they were such ignorant, how they received one year later the Nobel Prize? To my mind, the distortion of Paulescu's work was deliberately done to distract the attention from the inconvenient fact that the discovery of insulin had already been achieved.

Referring to the Macleod, Bliss stated: "*Perhaps as commonly happens with even the best informed professors, Macleod had not yet read Paulesco.*" ("Discovery of Insulin" by Michael Bliss, page 208). Macleod itself contradicted this claim in 1926 in the book "*Carbohydrate metabolism and insulin*", referring to the work carried out in Toronto, he said:

"While this work was in progress in Toronto, a paper by Paulesco came to our notice and after it was complete, one by Gley. Paulesco's researches were communicated at the meeting of Reunion Roumaine de Biologie in spring of 1921 in which he described the effects produced by intravenous injection of sterile pancreatic extracts on the percentage of sugar, of acetone bodies and of urea in the blood and urine of depancreatized dogs. Typical observations are shown in tables 1, 2 and 3.

There can be no doubt that all three substances became markedly reduced in amount in both blood and urine, as a result of the injection. The results were the same whether the injection was made into a branch of the portal vein or into the jugular vein. The effects were noticeable in one hour following the injection, attained their maximum in two hours and passed off in twelve hours. They varied with the amount of gland present in the injected extract. Paulesco also observed that the blood sugar as well as the blood urea in a normal dog became lowered by the injection. No observations are recorded of the behaviour of the respiratory quotient or of the glycogen content of the liver and no evidence is given that the general symptoms of diabetes were lessened or the life of the animal prolonged."

In fact, Macleod read Paulescu's paper during his holiday in Europe, and from that time he realized, for the first time, that Banting's idea to prepare a pancreatic extract from duct-ligated pancreas, to be used in diabetes, could work. Paulescu's papers convinced him that the internal secretion is a reality. So, returning to Toronto in 1921, he took the initiatives, asking Collip to join the team as soon as possible. Already in November 1921, he sent a letter to Joslin saying:

“It is true that we have been doing work on the influence of Pancreatic extracts, which has yielded most encouraging results, but I would rather hesitate to attempt the application of these results in the treatment of human diabetes until we are absolutely certain of them. Dr. Banting and Mr. Best who have been doing this work, are to report their findings at the meeting of the Physiological Society at New Haven, by which time we expect to be in a position to come to a definite conclusion. I may say privately that I believe we have something that may be of real value in the treatment of Diabetes and that we are hurrying along the experiments as quickly as possible.” [Bliss, page 96].

This text demonstrates that the “race against time” had commenced in the summer of 1921, when Macleod sent to Banting and Best the experimental method used by Paulescu and coincidentally, at the end of the summer, they totally changed their complicated protocol, adopting without any explanation, exactly the method used by Paulescu. It is our feeling that Macleod proposed to Banting and Best this new experimental protocol, without mentioning that this originated from Paulescu’s work. Macleod understood that the discovery of insulin had been done, but the utilization of this discovery in the treatment of diabetes was not reported by Paulescu. Thus, when he invited Collip to join the team, his aim was specifically the purification of the pancreatic extract for the purpose of administering it in man. Banting lacked the finesse to understand their real position in this “insulin affair”, but he instinctively felt that the clinical application would be a very important step. It was not by chance that in the period of December 1921 and February 1922, Collip approached those aspects of Paulescu’s work, such as the antiketogenic effect of the pancreatic extract which Banting and Best had not studied. To their surprise, Macleod brought up for discussion these effects at the Physiology Reunion in New Haven on 30 December 1921, when the Canadian team orally presented the results of their work till then.

THE PATENT OF “PANCREINE”: 10 APRIL 1922

Since 1920 Paulescu thought that his new hormone, Pancreine, is also the **key element** in the treatment of diabetes, and he considered preparing a patent for the new product. Soon after the end of his experiments, Paulescu applied in Bucharest on 10 April 1922 for a patent named „*Pancreine and the process of its fabrication*” [27], the clearest proof of his aim to use the hormone in the treatment of diabetes. The content of this patent was based on a

parallel work done by him in a biochemistry laboratory. The reader is invited to observe the clarity of this text, its biological expertise of the author and its sense of responsibility for a product intended to be used in human being.

His efforts in 1922 were directed towards the technical problems related to the purification of his pancreatic extract. After many trials, he obtained a rather highly purified extract which he described in his application for a patent. The approval of its application would come on 10 April 1922 as patent no. 6254. Here is the full text of this patent which includes, in the method of its purification, two additional steps to the procedure he used in obtaining the extract in 1921. For this reason, the solution containing the extract was extremely powerful and could produce very low blood sugar levels, sometimes below the detection limits of the methods used by Paulescu (called “*aglycaemia*” by him). Later, Collip made the same observations using his purified extract prepared at the beginning of 1922. Bearing in mind the modest facilities at his disposal, Paulescu’s observations highlight even more the accuracy of his methods and the keenness of his spirit of observation.

PANCREINE

AND THE PROCESS OF ITS PRODUCTION

“I give this name to the active principle discovered by me in the extract of the pancreas. See: Paulescu Recherché sur le rôle du pancrèas dans l’assimilation nutritive, in Archives Internationales de Physiologie, Liège, Vol XVII, p. 85.

This substance has remarkable properties which, when injected into the blood of an animal rendered diabetic by extirpation of the pancreas produces:

- a. a diminution or even a transient suppression of hyperglycaemia and glycosuria;*
- b. a diminution in blood and urinary urea;*
- c. a diminution in blood and urinary acetone;*

In order to isolate Pancreine as much as possible from other proteins, I proceed in the following manner:

Observing strict antisepsis, I take a certain portion of the fresh pancreas from a recently sacrificed animal.

The gland is well minced in a Broyeur Latapie machine and sterilised in an oven.

To this minced pancreas is added ten times its weight in distilled water, after which I shook it many times and introduced it into a cooler.

After several hours, 6 - 24, the minced pancreas is filtered through a sterile double gauze compress in order to remove the very voluminous solid parts.

The filtrate, which is cloudy, is more or less rose in colour and to it is added pure hydrochloric acid 10 pp 1000, which brings on an abundant protein precipitate.

The grey precipitate is separated with a sterile gauze filter and, as the liquid is acid, it is neutralised using caustic soda.

Thus a new and abundant protein precipitate is produced.

The new precipitate is separated using a Berzelius paper and sterilised.

The filtered liquid is clear and transparent and still gives a protein reaction.

Finally, the volume of this liquid is reduced by evaporation at a temperature which must not exceed 50°C.

In order that pancreine be used in the treatment of human diabetes, it must be prepared in large quantity, which requires a lot of capital.

In addition, it is absolutely necessary that strict measures for antiseptics be observed in making this preparation.

At the same time, all the physico-chemical requirements of the process must be carefully observed especially that the liquid temperature does not exceed 50°C.

If these diverse steps are not very rigorously followed, the medicine may become either a focus of infection, thus bringing about a disaster or it loses its **physiologic action**. In order to ensure the fulfilment of this fundamental condition of preparation as well as to maintain the scientific standard of the product **thought it necessary** to demand a patent.

CLAIM

I claim the invention of the organic pancreatic product which, when injected into the blood, produces a diminution or even a transient suppression of the symptoms of diabetes."

Sgd N.C. Paulescu"

Because in 1920, Paulescu already anticipated that the pancreatic extract is the key in the treatment of diabetes, Canadians understood that the

utilization of pancreatic extract by Paulescu is imminent. This explains the acceleration of the work in Toronto, the fact that created an open conflict between Banting and Best, on the one side, and Macleod and Collip, on the other side. The key person in this step was Collip, who in less than 2 months, succeeded to purify the pancreatic extract. After a physical aggression of Banting against Collip, although frightened, he remained in Toronto several months, producing the first quantities of extract ("Isletin" called that time) with which all patients in the initial clinical trials were treated. As Bliss said (page 238): *"In later years, Collip was very reluctant to talk or write about the discovery of insulin, saying that the truth was to be found in the scientific publications and might emerge after they were all dead."*

In an interview for the Star in September 1922, he said: *"There are some people in Toronto who felt that I had no business to do physiological work. Against this I would say that when I entered the collaborating group early in December 1921, it was with a view of putting my whole effort into pushing forward of the research irrespective of any water-tight compartments. The result was that when I made a definite discovery, my confreres instead of being pleased were quite frankly provoked that I had had the good fortune to conceive the experiment and to carry it out. My own feelings now in the matter are that the whole research with its aftermath has been a disgusting business."*

This bitter word is understandable because the full merit for utilization of insulin in Toronto belongs to Collip. Instead, Banting negotiated in the middle of September with Eli Lilly Company to transfer the production of insulin in order to remove Collip from the team, so that he could be the only owner of the method of insulin preparation. In fact, he robbed Paulescu's work, which had led to the discovery of insulin, and also Collip's purification method of pancreatic extract.

If the Nobel Prize was awarded for the clinical application of the discovery of insulin (which belongs to Paulescu) then Collip should have been taken into consideration for the Nobel Prize Award. In fact, the Nobel Prize was attributed to persons (Banting and Macleod), who did not deserve it.

HOW DID THE WRONGFUL AWARDED OF THE NOBEL PRIZE INFLUENCE THE DIABETOLOGICAL THINKING?

Since the destiny decided that Paulescu will be punished for his audacity to discover one of the most hidden secrets of life, his magnificent researches from 1916-1923 were neglected, the focus of attention being fixed on those

that received the Nobel Prize. They were the new prophets of science, and only their ideas should be considered. Since Banting and MacLeod thought in the old paradigm of diabetes, many years after the discovery of insulin, the study of the new hormone was strictly focused on its glucose lowering effects and diabetes continued to be identified with hyperglycaemia. In fact, hyperglycaemia remained for 100 years the only criterion for the diagnosis of this immense syndrome, conceived by Paulescu in a much larger biochemical perspective.

As we mentioned before, researchers progressively realised that diabetes is much more than a disorder of carbohydrate metabolism (*diabetes mellitus*), and they stated that diabetes can be equally considered as *diabetes lipidus* [14] (lipid disorders being indissolubly linked to this syndrome) and *diabetes proteicus* due to the important protein metabolism alteration [13]. The last term, proposed in a presentation of Marliss during the IDF Congress in Cape Town in 2006, confirmed in fact the vision that Paulescu had in 1912. Despite this, at that moment no one made reference to the papers of



Figure 2. N.C. Paulescu in 1928.

Paulescu and his discovery of the anti-diabetic hormone. It is currently well known that the numerous attempts to publish the truth in the major diabetological journals (*Diabetes*, *Diabetologia*, *Diabetes Care*) faced a cunningly justified refusal: the above mentioned journals do not have the aim to publish historical points of view. This long lasting resistance is illustrated by the major difficulties faced between 1969-1971 by the Scottish historian Ian Murray (1899-1974), an important personality of diabetology during those times, who published the first papers that demonstrated the priority of Nicolae Paulescu in the discovery of insulin [28-30]. This attitude was manifested by an international personality absolutely impartial, pleading in favour of Paulescu only in the spirit of fairness that should be present in the scientific community. Ian Murray makes a breach in the embargo on discussing the conflicts inside the Canadian group that took place between 1922-1923. Despite the fact they were mentioned only with silence, details regarding the violence of

Banting towards the other members of the team were well known. In time, the breach created by Ian Murray became a large rift through which, nowadays, flows the large river of truth.

However, with the occasion of celebrating 75 years from insulin discovery, Korec R. presented a paper showing that using a protocol for obtaining an active pancreatic extract (similar to the patent of Paulescu) in a diabetic animal model, the administration of Pancrein had a significant effect on glycemia [31].

The resistance of the North-American diabetologic community regarding the interventions of Prof. Ion Pavel (1897-1991) in favour of Paulescu between 1971-1973 were even stronger and, I could say, more and more refined. This can be clearly seen from the correspondence on this topic of Prof. Pavel with different members of the international diabetes societies of those times. He published this correspondence in two books [32,33]. His disappointment can be illustrated by his conclusion from the cover of the last book:

“I devoted all my efforts for the last two decades to the recognition of the priority of N.C. Paulescu in the discovery of insulin. Analysing today the circumstances that for 65 years wrong this great scientist, I find out that, finally, this injustice is the result of a grievous lack of scientific ethics”.

If there still is a lot of scepticism on the *total reconsideration of the truth regarding the discovery of insulin*, our optimistic point of view was already expressed in 1996, with the occasion of the 75 year anniversary of insulin discovery [2]. We were, and we still are convinced that time is on Paulescu's side and it favours him. This is proven by the fact that at the International Diabetes Federation Congress in Montreal in October 2010, Prof. Alberto de Leiva, in a wonderful lecture regarding the history of diabetes, put in a correct light the outstanding contribution of Paulescu's work in the insulin discovery from 1921. In addition, in two books regarding the history of diabetes published in 2009 [33,34], as well as in an important Diabetes Textbook published in the UK in 2010 [35], the results of Paulescu with their true relevance and his portrait appear constantly before those of the Canadians.

CONCLUSION

Controversies regarding the scientific discovery cannot be impartially established, without taking into account two objective criteria: (1) the date of publication in international journals, and (2) the scientific value of the

data. Using these criteria, discovering of the antidiabetic hormone, finally called the “insulin”, and describing all its physiological properties was done by N. C. Paulescu with more than half a year before the Canadian team. The therapeutic application of this discovery was successfully implemented on 23 January 1922 using a purified pancreatic extract prepared by Collip.

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SAŽETAK

Svrha je ovog članka opisati istraživanje Nicolaea Constantina Paulescu a istaknuti njegovu ulogu u otkriću inzulina.

Metode: Temeljito smo pregledali literaturu i istraživanja u rumunjskoj akademskoj arhivi kako bismo pronašli odgovarajuće reference.

Rezultati: N. C. Paulescu je 1912. analizirao kliničke i biokemijske promjene kod pacijenata s dijabetesom i kod pasa nakon izvođenja pankreatektomije, koji su osim hiperglikemije i glikozurije (metabolizam ugljikohidrata) zabilježili i promjene u metabolizmu lipida i proteina. Godine 1916. Paulescu je započeo eksperiment s ekstraktom gušterače dobivenim njegovom izvornom metodom, koji je intravenski ubrizgan u pse koji su bolovali od dijabetesa. Rezultati njegovih prvih eksperimenata pokazali su: "Ekstrakt gušterače ubrizgan u perifernu venu proizvodi: 1. smanjenje, pa čak i privremenu supresiju dijabetičke hiperglikemije, koja može biti zamijenjena hipoglikemijom; 2. smanjenje ili čak privremenu supresiju glikozurije; 3. smanjenje uree u krvi; 4. smanjenje uree urina. Drugim riječima, intravenozna injekcija ekstrakta gušterače ima za posljedicu nestanak simptoma dijabetesa. Smanjenje dijabetičkog sindroma počinje odmah nakon ubrizgavanja, a maksimum doseže nakon dva sata – i traje oko 12 sati." Zaključio je: "Ovo otkriće, koje baca novo svjetlo na patogenezu dijabetesa, daje nam i ključ za liječenje ovog sindroma." Paulescu je 1921. objavio svoje podatke u dva izvanredna francuska časopisa i to osam mjeseci prije prve publikacije Bantinga i Besta u veljači 1922. Jasno je da je inzulin otkriven u Europi.

Zaključak: Paulescu je smatrao da je novootkriveni hormon, nazvan pancrein, ključni element u liječenju dijabetesa, ali njegovo izvanredno istraživanje nepravedno je zanemareno.

Ključne riječi: otkriće inzulina; medicinska etika; kronologija događaja i kontekst; eksperimentalni dizajn.