

## N.C. PAULESCO: 105 YEARS FROM THE DISCOVERY OF INCRETINIC EFFECT

Constantin IONESCU-TÎRGOVIȘTE<sup>1,2</sup>, Alice DULA<sup>2</sup>, and Cristian GUJA<sup>1,2</sup>

<sup>1</sup>“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

<sup>2</sup>“N. C. Paulescu” National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

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The last antidiabetic oral or injectable drugs called “gliptine class” had as a starting point the discovery of “incretine effect” mainly induced by proximal (duodeno-ileal) gut which produce Glucose dependent Insulinotropic Peptide (GIP) and distal gut (last ileon and first part of colon) which produce Glucagon Like Peptide 1 (GLP-1). The last one has a short time of action, being inactivated by the enzyme Di-Peptidil Peptidaze 4 (DPP-4). The stimulation of insulin secretion can be obtain either by the agonist of the  $\beta$ -cell receptors of GLP-1 or by inhibition of DPP-4 prolonging such the physiological action of GLP-1. For us, is rewarding to know that the first description of the incretine effect, as a condition for the utilization of glucose by the liver for its transforming it in glycogen, only by its oral administration, which has been done by Paulesco in 1911. This effect is not observed if the administration of glucose is made directly in the portal vein.

Recently, Holst, Gribble, Horowitz and Rayner, in a paper intituled “*Role of the gut in glucose homeostasis*” (Diabetes Care 39:884–892, 2016) referring to the incretin effect, begins their review with the following statement: “*One of the ways to illustrate the role of the gut in glucose homeostasis is to compare the fate of glucose that has been administered orally or infused intravenously*”. A such thinking had Paulesco when in 1911 started the long cycle of experiments dedicated to the role of the gut in the acumulation of glycogen into the liver<sup>1</sup>. It is interesting to know that after the crucial discovery of liver glycogen by Claude Bernard (1813–1878) in the middle of 19<sup>th</sup> century, many researchers has been involved in the study of fate of the ingested foods reflected in the acumulation of glycogen into the liver ( in some experiments also in skeletal muscles and in the myocardial tissue). It is amazing to see how many researches (critically analysed and commented by Paulesco in his paper from 1911)<sup>1</sup>, tried to clarify the importance of gut in the glycogenic function of the liver. This is the reason for he to consider that a such difficult problem should be treated in a manner that respects all the rules of “*physiological experimentation*”. Otherwise the methodology of a such physiological

experiments was presented by him to the young students yearly in one of his inaugural lesson at the Faculty of Medicine from Bucharest between 1901 and 1931 (the year of his death) and peobably later by his successors.

After 42 experiments which he performed and are presented with all details in four tables: in *Table 2* (15 experiments), in *Table 3* (8 experiments) and in *Table 4* (17 experiments), Paulesco finds that direct injection of glucose in portal vein or in an afferent vein of it, does not lead to the storage of glycogen into the liver.

The conclusive data of all this experiments is given in the *Table 1* including only 2 cases folowed by his clear final conclusion: “*In our experimental conditions, we can not observe the production of glycogen in the liver, by carbohydrates (glucose, levulose, maltose, or dextrans), when are injected directly in the portal vein. On contrary, these injections have an effect of decreasing up to zero the content of glycogen in the liver*”<sup>1</sup>.

In this state-of-art paper<sup>1</sup> Paulesco gives the first indirect experimental proof clearly showing that the intraportal administration of various carbohydrates are not able to be stored in the liver as glycogen. On contrary, in the six next publications in 1913<sup>2-7</sup> he sistematically demonstrated that the oral administration of glucose<sup>4</sup> and of proteins<sup>5</sup> leads to a rapid storage of glycogen in the liver. On contrary,

no glycogenogenetic effect was observed after administration of fatty acids, glycerin or ethilic alcohol<sup>7</sup>. The explanation of the differences in glucogenetic effect has been later explained by the neoglucogenetic effects from proteins (from so called glycogenic amino acids)<sup>5</sup>, but not from fatty acids<sup>6</sup>.

In addition he studied also the glycogen after the extirpation of the pancreas<sup>8</sup> suggesting that the

internal secretion of the pancreas (called by him "Pancreine") which has been already discovered in 1916 and due to the first great war has been published for the first time in his "*Traité de Physiologie Médicale*", vol. 2, 1920. The main publications of his data in international journals has been possible only 5 years later<sup>9</sup>, however with 8 months before the first publication of Banting and Best in February 1922.

Table 1

The results of the experiments carried out on two dogs

Experiments	The weight of dog	The weight of liver	The period of starving	The quantity of extrated serum	The proportion of glycogen	The duration of injection	Glycogen	
							Before Injection	After Injection
2	Gr	Gr	Days	Cc	Gr	h. m		
	3170	153	10	2000	3	1.30	0	0
	13700	441	8	3000	9	0.30	0	0

It is now clear that through this experimental observations, Paulesco discovered also "*the incretinic factor*", of course not using this term, which was introduced half a century later by Nauck in 1968<sup>14</sup>, based on the data obtained by McIntyre *et al.* (1964)<sup>11</sup> and Perley and Kipnis (1967)<sup>13</sup>. These last groups of researches observed a difference in the insulin response after an oral *versus* intravenous glucose tolerance test. Using the oral administration of glucose the stimulation of insulin secretion is higher than the intravenous administration<sup>11-13</sup>. In healthy subjects, the oral administration of glucose induces a 2 to 3 fold higher insulin response than the intravenous route as result of the actions of the incretine hormones.

In 1987, Kneymann *et al.*<sup>15</sup> discovered the intestinal hormone Glucagon-Like Peptide 7-36 (GLP-1) containing 29 amino acids, interpreted as *a physiologic incretine in man*. Besides this hormone, which proved to be produced in L distal intestinal cells, in duodenal and upper ileal mucosa, K cells produce from proximal (duodeno-ileal gut) another incretine hormone named *Glucose-dependent*

*Insulinotropic Polypeptide* (GIP) containing 43 amino acids<sup>16</sup>. Although quantitatively GLP-1 is lower than GIP secretion, the major incretine effect mediated by the stimulation of the insulin secretion is due to GLP-1 molecule, through their specific receptors present on the pancreatic  $\beta$ -cells membrane<sup>16</sup> (Table 2).

In type 2 diabetes, one of the mechanisms involved in the appearance of  $\beta$ -cell secretory dysfunction is that of decreased half-life of GLP-1 by the hyperactivity of Di- Peptidyl Peptidase (DPP4) enzyme. The inhibition of DPP-4 was found to increase the stimulation of the  $\beta$ -cell by the GLP-1. In such way in the last two decades have been developed two classes of drugs: the GLP-1 agonist and DDP4 inhibitors, generically named *glyptins* with many members<sup>18</sup>.

The first GLP-1 agonist, Byetta, have been approved in 2005 in USA and in 2007 in Europe and, Liraglutide (Victoza), the first GLP-1 analogue, has also been approved in Europe in 2009 and in USA in 2010 The Di-Peptidyl Peptidase

inhibitors (glyptins) were introduced gradually on market in 2007 Sitagliptin (Januvia), in 2008

Vildagliptin (Galvus) and in 2009 Saxagliptin (Onglyza) (Table 3)<sup>17-20</sup>.

Table 2

The intestinal hormones produced along the digestive tract

GUT HORMONES	Place of secretion of gut hormones
GLP-1 (Glucagon Like Peptide-1)	L-cells
GIP (Glucosozoddependent Insulinotropic Protein)	K-cells
Peptide YY	L-cells especially ileon and colon but also along the digestive tract
Glucagon	$\alpha$ 2 cells from Langerhans islets
Gastrin	Stomach
Secretin	S-cells of the duodenum and stomach
Cholecystokinin	Enteroendocrine cells (including L-cells) in the duodenum
VIP (Vasoactive intestinal polypeptide)	Gut, pancreas and suprachiasmatic nuclei of the hypotalamus in the brain
PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide)	Gut, pancreas

A specific agonist of GIP receptor on  $\beta$ -cells has been not available until now for use in human diabetes<sup>28-35</sup>.

The relative contribution of GLP-1-mediated *versus* non GLP-1-mediated mechanisms by which the inhibitor DDP-4 lower glycemia, stimulate insulin and suppress glucagon in patients with type 2 diabetes using various inhibitors of DDP-4 after mixed meal, show that the positive effect is 40% GLP-1 mediated and 53% non GLP-1 mediated<sup>19</sup>. It has been shown that the treatment with GLP-1 agonists of obese patients with only impaired glucose tolerance suggests their role in the prevention of type 2 diabetes<sup>21,22</sup>.

A short recent history of *incretinic axis* (which include also a central station in hypothalamic region) is given in Table 3. The time is coming to remember that the Paulesco's careful experiments carried out between 1911–1913 on healthy dogs in two stages: in a first stage the dogs were starved to the complete depletion of hepatic glycogen stores,

confirmed by the quantitative dosage of glycogen using Pfluger's method<sup>2</sup>. After that, in a second stage, to these dogs was administered exclusive food: either carbohydrates, or proteins, or fats. The results, which are entered in the patrimony of liver physiology, were clearly summarized as such: the restoration of glycogen is fast and maximal after feeding with carbohydrate; the effect is also good but a little slower, by the administration only protein; on contrary, administration of only fats does not produce the restoration of glycogen.

The first GLP1 agonist, Byetta, have been approved in 2005 in USA and in 2007 in Europa and, Liraglutide (Victoza), the first GLP-1 analogue has also been approved in Europa 2009 and in USA in 2010. The DPP-4 inhibitors (glyptins) were introduced gradually on the market in 2007 Sitagliptin (Januvia), in 2008 Vildagliptin (Galvus) and in 2009 Saxagliptin (Onglyza)<sup>23,24,27,32</sup> (Table 3).

Table 3

History of the "incretine effect" and drug developments for clinical application

1911, N. C. Paulescu<sup>2</sup>:

The intraportal administration of glucose and other carbohydrates has any effect on liver's glycogenic function in starved dogs, in contrast with the oral carbohydrates administration with a powerful and rapid accumulation of glycogen in the liver. Paulescu advanced also hypothesis that the internal secretion of the pancreas may have an important role in the glycogenic accumulation into liver and in the other peripheral tissue (muscles, myocardium)<sup>2</sup>.

1964, McIntyre<sup>12</sup>; Erlick<sup>13</sup>; Perley (1967)<sup>14</sup> noted that the administration of the same quantity of glucose intravenously gives a lower insulin response than that obtained after its oral administration.

1974, Brown JC<sup>15</sup>; Turner DS<sup>16</sup> Discovery of GIP (Glucose dependent Insulinotrop Peptide) called initially Gastric Inhibitory Popypeptide (GIP)

Table 3 (continued)

1984,	Nauck Incretin effect reduced in Type 2 diabetes <sup>17</sup>
1987,	Kneymann <sup>18</sup> ; Discovery of GLP-1
2005,	Exenatide (Byetta) the first GLP-1 agonist with administration 2 times/day (approved in 2005 in USA and in 2007 in Europe)
2006,	Sitagliptin (first DPP-4 inhibitors)
2008,	Vildagliptin (Galvus)
2009,	Liraglutide (Victoza)
2009,	Saxagliptin (Onglyza)
2012,	Exenatide (Bydureon) with administration 1 time/week

In a historical perspective we can observe that the outstanding observation of Paulesco has been totally neglected half of century being rediscovered after 53 years by McIntyre<sup>11</sup>, Erlick<sup>12</sup> and another 22 years since Nauck who used the term “*incretine effect*”<sup>14</sup>, identified in the secretory function of digestive tract. For the therapeutically application of the agonists or inhibitors of some hormones secreted by specific intestinal cells, needed another 43 years.

This is how the oversight of an outstanding experimental observation, can dramatically influence the progress in the understanding the pathogenesis of diabetes and the production of new and efficient drugs for type 2 diabetes. Regrettable is the fact that such an oversight of a great discovery is a penalty for the later development of the scientific field involved. Anyhow, we hope that for now the history of incretine effect will start with the Paulesco’s discovery, the man who later discovered insulin almost one year before the Canadian team<sup>23</sup>. Along the time this discovery were attributed either to Banting and Best or Banting and McLeod, or in the last time McLeod and Collip. The last one was the biochemist who succeeded to purify the pancreatic extract and that extract was the only one used in 1922 in Toronto. Banting and Best has nothing to do with the introduction of the purified extract in the treatment of diabetes<sup>23</sup>. In retrospect, a correct decision based on written document in 1923 should be the awarding Nobel Prize to Paulesco & Collip<sup>27</sup>.

Shortly, insulin has been discovered as physiological hormone by Paulesco in 1921 (under the name “Pancreine”) and the clinical utilization of the pancreatic extract has been done by Collip in 1922<sup>23</sup>. McLeod used in May 1922 the term “insulin”, suggested in 1909 by the Belgium physiologist Jean de Meyer. So, the antidiabetic hormone has been discovered in Europe and even its name has been done by a European scientist<sup>28</sup>.

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