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Glycolic Ferment: The Work of Victor Barral and Raphaël Lépine

Jaime Wisniak*

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Abstract

Etienne Victor Barral (1860–1938), a French physician and chemist, and Raphaël Lépine (1840–1919), a French physiologist, discovered after extensive research that the assimilation of sugar in the blood was carried by a glycolytic ferment, produced mainly by the pancreas, without accumulation. Their work and consequences led other researchers to isolate the glycolytic agent known as insulin afterwards.

Key words: Blood, Diabetes, Ferment, Glycolic ferment, Glycolysis, Insulin, Medicines, Pancreas, Pathology, Saccharification, Sugar, Urine.

1. Sugar in blood - historical review

Diabetes is an illness that has affected humans and other living organisms since ancient times. It took a long period of time until its cause was discovered and methods were developed to alleviate its consequences.

Barral (Appendix 1) alone, or with his mentor Lépine (Appendix 2), published many papers on the subject (Barral, 1890ab, 1892; Lépine, 1889, 1890, 1909; Lépine and Barral, 1890abc, 1891a-m, 1892a-e). In the introduction to his doctoral thesis, Barral gave a detailed description of the history of diabetes and the contribution of several scientists (Barral, 1890a). Lépine afterwards completed this review (Lépine, 1909) and divided the history of developments into four periods: (1) up to 1775, (2) 1775 to 1847, (3) 1847 to 1877 (the work of Claude Bernard), and (4) 1877 to 1889. A summary of the most important landmarks follows. Lépine wrote that in ancient times it was known that certain illnesses were accompanied by the release of an excessive amount of urine; Aulus Cornelius *Celsus* (c. 25 BCE–c. 50 CE) believed that this phenomenon was due to the fact that drinks traversed the body without being retained, and assigned it the name διαβήτης. He also mentioned that these patients suffered from unquenchable thirst and fatigue. Aelius Galenus (130–210) admitted that diabetes was due to a weakness of the kidneys, which had lost their ability to retain liquids, a doctrine that was afterwards adopted by Alexandre of Tralles (525–605) and Avicenna (980–1037). Afterwards, Vittore Trincavelli (1496–1568) gave us proof that urine was composed by the unchanged drink with the fact that it tasted the same as its infusions, and added that the stomach was also part of the defect. Paracelsus (1493–1541) added a new idea: the blood contained a salt that when eliminated by the kidneys resulted in polyuria. Several physicians such as Jean Fernel (1497–1558), Thomas Willis (1621–1675), Felix Plater (1536–1714), and Richard Mead (1673–1754) took opposing positions regarding the organ or fluid responsible for the malady.

In the year 1776, the physician Mathew Dobson (1732–1784) reported the presence of sugar in the

* Department of Chemical Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel-84105. Email: wisniak@exchange.bgu.ac.il

urine of a deceased 33 years old male, suffering from diabetes. According to Dobson the urine of all his diabetes patients had a sweet smell and taste and was almost perfectly transparent and colorless (Dobson, 1776). Left to itself, it resulted in a spontaneous fermentation and deposited a white loose precipitate, accompanied by release of air bubbles. Eventually, a thin layer covered the surface of the urine, resembling the one on top of fermenting liquors; it then changed to a putrid and offensive material. Upon boiling, the fresh urine showed no sign of coagulation; the dry residue was a granulated white cake, smelling sweet like brown sugar, not reacting with sulfuric acid elixir, and effervescing with concentrated sulfuric acid, accompanied by release of HCl. According to Dobson, the results of his experiments indicated,

> That the fluid that was separated by the kidneys of the patient showed little or none of the sensible properties of urine but contained a substance that readily passed through the vinous, acetic, and putrefactive fermentations.

It appeared that a

> considerable amount of saccharine matter passed of by the kidneys...This saccharine matter was not formed in the secretory organ but previously existed in the serum of the blood...These results led to question if diabetes should be considered as a species of imperfect digestion of assimilation.

Hence, Dobson attempted to treat his diabetic patient with a variety of remedies (i.e. rhubarb, opium, and cantharidin). He hoped that it would help his patient to better digest his meals. These means proved unsuccessful (Dobson 1776; Barral, 1890a). Afterwards, Pierre-François Nicolas (1743–1816) and Gueudeville looked unsuccessfully for sugar in the blood by coagulating it slowly in the presence of air and subjecting it to a series of distillations, without understanding that the latter led to the destruction of the sugar (Nicolas and Gueudeville, 1802). William Hyde Wollaston (1726–1828) investigated the presence of sugar in blood by completely coagulating it by heat in the presence of a small amount of HCl, and evaporating the remaining liquid to dryness. The solid residue was found to contain only crystalline salt (NaCl) and no sugar. He verified this result by treating the residue with nitric acid: the resulting matter contained only sodium nitrate, which crystallized without foam or blackness. The presence of sugar would have resulted in foaming and blackening under the action of heat. Wollaston believed that if sugar was present in the blood it had to be in an amount lesser than 1/30 of the serum. Wollaston concluded that this sugar was formed in the stomach by a process of imperfect assimilation and from there it travelled by some conductive means to the bladder, without passing through the general system of blood vessels (Wollaston, 1811). Louis Nicolas Vauquelin (1763–1829) and Pierre Salo-mon Ségalas (1792–1875) detected the presence of sugar in the urine of a woman affected by diabetes and failed to find it in a 24-hour old blood sample (Vauquelin and Ségalas, 1825).

In 1815 Michael Chevreul proved that urine sugar was chemically identical to grape sugar and William Prout confirmed his result (Chevreul, 1815; Prout, 1817). In 1844, Louis Mialhe (1806–1888) published a note describing his theory about the causes of diabetes, which he had perceived as the result of some doubtful chemical analysis of sugars (Mialhe, 1844). Contrary to the claim of many chemists he had found that grape sugar (glucose, or diabetes sugar) was unable to reduce cupric oxide, under hot or cold conditions. Glucose acquired this capability only after being chemically changed by an alkaline substance, free or as carbonate. Mialhe experiments indicated that carbohydrate foods such as grape sugar, gum, starch, or dextrin, were assimilated only after the alkalis of the blood had transformed them into new products; among them was an unknown one having a strong deoxygenating power, able to easily reduce ferric oxide to ferrous oxide, all ferric salts to ferrous salts, etc. As a result, the urine of a healthy person would not contain sugar. If this

transformation did not occur, the original materials would remain unchanged and, consequently, would be excreted by the kidneys. Diabetes should then be considered a defect of assimilation; sugar, instead of promoting the organic chemical changes taking place in the body would act as a foreign body, which the economy would try to eliminate persistently (Mialhe, 1844).

Barral remarked that in 1847 the French physiologist Claude Bernard (1813–1878) had begun a series of investigations that led him to prove that sugar was normally present in the blood, separately of digestion phenomena and independent of the nature of the food (Bernard, 1848, 1849, 1851, 1853, 1857). Bernard used different methods for detecting and quantifying the amount of sugar in the blood. Initially he collected the blood in boiling water and dosed it using Fehling's liquor; unfortunately this procedure took too much time and never gave a liquid absolutely clear. He also tried to bleach the liquid with animal carbon but soon realized that this material absorbed the sugar. In due time, he found that the best analytical procedure was based on heating the blood with sodium sulfate and treating with alcohol. A comparison of the blood of the portal vein (conveying blood to the liver from the spleen, stomach, pancreas and intestine) with that of the hepatic veins led him to discover the influence of the liver on the distribution of sugar in the different parts of the circulatory system and the notion of the hepatic glycogen. The blood of hepatic veins was rich in sugar that disappeared almost completely in the blood of the portal vein. Bernard realized that the blood sugar was destroyed by fermentation, not as with beer yeast but by the lactic fermentation. Lactic acid was then burned in the tissues and transformed into CO_2 and water. This fact explained the partial disappearance of part of the oxygen in the blood and its replacement by CO_2. Thus, two successive fermentations were followed by an oxidation: the first oxidation was the formation of sugar from albuminous substance, the second was its transformation into lactic acid, followed by oxidation. Bernard also found that sugar passed into the urine only when it contained more than 3/1000 parts; diabetic blood was alkaline, and the entire organism was impregnated with sugar. According to Bernard, blood was destroyed continuously at body temperature and kept longer at laboratory temperature. Phenol, sodium sulfate, and acetic acid retarded the decomposition. The level of sugar in blood increased with a sweet or feculent diet and decreased with inanition and with certain pathological states (Bernard, 1848, 1849, 1851, 1853, 1857).

Afterwards, Auguste Chauveau (1827–1917) made some additional important discoveries: (1) sugar did not disappear from the blood, even after a long fastening; (2) arterial blood always contained the same proportion of sugar, independent of the point where the sample was taken; (3) in animals fed with meat, the hepatic blood contained more sugar than in the other vessels; (4) pure lymph was always sugared, even after a long abstinence; (5) the blood from both hearts contained the same amount of sugar; (6) blood sugar was not destroyed in the lungs, and (6) blood became loaded with sugar after passage through the liver (Chauveau, 1856, 1857).

In 1877 the British physiologist Frederick William Pavy (1829–1911) found a small difference in the amount of sugar present in arterial blood and in vein blood. Anesthesia increased the amount of sugar and after death it did not decrease as fast as claimed by Bernard (Pavy, 1878). In 1879 Paul Cazeneuve (1852–1934) proved that Bernard's analytical method of defibrinated blood was slightly in error due to the presence in blood of substances that reduced Fehling's liquor (Cazeneuve, 1879). Between 1886 and 1887, Chauveau and Maurice Kaufmann studied the effect of muscle activity upon the level of sugar in blood. They found, in general, that the level of sugar in the blood leaving an organ was lower than

the level of the blood entering the organ. The change in level was clearly connected with the processes of combustion and production of heat. Chauveau and Kaufmann reached this conclusion by comparing arterial blood with vein blood and the amounts of oxygen absorbed and of CO_2 produced in the capillaries. These analyses were conducted in the masseter muscle and in the parotid, two organs belonging to the same functional group. Their results indicated that the production of CO_2 in the muscles was about five times larger that in the glands, and the transformation of arterial and vein blood was accompanied by a destruction of glucose five and a half times lower than that produced in the muscle. In addition, when these two organs were physiological active, the amount of blood passing through them was three times larger than the amount passing when they were at rest (Chauveau and Kaufmann, 1886, 1887ab). In 1889 Friedrich Joseph von Mering (1849–1908) and Oskar Minkowski (1858–1931) found that total extirpation of the pancreas of a dog, rabbit, and pigeon, led to their urine containing sugar and acetone and that the corresponding diabetes persisted, without change, until the death of the animal. The animals also suffered of polyphagia and polydipsia (Mering and Minkowski, 1889).

2. Dosage of sugar in blood

Many different procedures had been proposed for the dosage of sugar in blood (Barral, 1890ab). The simplest and oldest consisted in receiving the blood in acidified boiling water and pressing the coagulum with a cloth. The resulting liquid was never clear enough to allow determining the amount of sugar by means of Fehling's liquor. Clarification by means of egg white was inappropriate because this substance also contained sugar. Bertrand's dosage procedure had proven to be the best: Twenty-five cm^3 of blood were mixed with 25 g of crystalline sodium sulfate and heated in capsule of known weight. The cooking resulted in a sparkling coagulum that later turned into a black spongy substance accompanied by a large amount of liquid. Distilled water was then added to restore the original weight and the resulting mixture pressed out. The amount of sugar was then determined in the resulting liquid. According to Bernard, urates, which reacted with Fehling's liquid, were retained by the blood coagulum. The heating process also eliminated the chloroform present in the blood of anesthetized animals. The use of sodium sulfate was enough and sufficient to obtain a liquid that permitted easily the dosage of sugar with Fehling's liquid, added by means of a graduated pipette (Barral, 1890ab). Lépine and Barral modified Bertrand's method to make it faster and more accurate: the blood (25 g) was poured on an equal weight of boiling acidified sodium sulfate (25 g) and the resulting liquid (38 cm^3) filtered and titrated with Fehling's liquor, taking care that the latter was mixed with an equal amount of sodium or potassium hydroxide to dissolve the resulting copper oxide. Using these amounts led to the formula $x = 7.6/n$ where x was the amount of sugar contained in 1000 g of blood and n was the number of cm^3 employed in the titration. Lépine and Barral found that instead of mixing the Fehling liquor with concentrated NaOH or KOH it was better to add only 5 to 6 cm^3 of a mixture of freshly prepared solution of alkali of 10% concentration by weight, and 40 to 50 cm^3 of distilled water. This assured the total solution of the precipitated copper oxide and avoided the error introduced by the fact that part of sugar was destroyed by the glycogenic ferment during the initial heating process (Barral, 1890ab).

3. Pancreatic diabetes

In 1889 Lépine suggested that pancreatic diabetes was caused by an insufficiency of ferments (Lépine, 1889). A year later, Lépine and Barral performed the following experiment to support this theory (Lépine and Barral, 1890a): A

dog was kept fasting for 36 hours and then its pancreas was completely removed. Previously, another similar animal of the same size was kept on an empty stomach for 60 hours. Twenty-four hours afterwards, both animals were sacrificed by arterial hemorrhage and all their arterial blood collected. The amount of sugar in the blood was determined (using Fehling's liquor) at three different time points: (1) on leaving the artery, (2) half-an-hour later, after defibrination, and (3) fifteen hours later the blood was left for the night, at the temperature of the laboratory. The results were as follows (sugar in 1000 g of blood):

Sample	Healthy dog	Dog without pancreas
1	1.17	3.30
2	1.10	3.23
3	0.72	3.04

These results showed again not only the large amount of sugar present in the blood of the dog deprived of pancreas but also its small decrease with time. During the same length of time the blood of the healthy dog had lost 0.45 g while that of the affected animal had lost only 0.26 g (Lépine and Barral, 1890a). Lépine and Barral also added the same amount of starch to both samples of fresh blood and found that after 15 hours, the amount of additional sugar produced was 3.37 g for the healthy dog and 2.37 g for the dog deprived of pancreas. This result proved clearly that the blood of the healthy dog contained a larger amount of glycolytic ferment. Assuming the value 1 for the amounts of sugar present at the end of 15 hours (see above table), it meant that the amount of sugar produced by each dog were 337/72 = 4.48 and 237/304 = 0.77 respectively; in other words, a difference of about *six times* (Lépine and Barral, 1890a).

4. The Glycolytic Ferment

In 1890 Lépine carried a series of experiments on dogs that had been deprived surgically of their pancreas and subsequently become diabetic. Injection of the chyle of another dog subject to the same ablation did not reduce the glycosuria. Further experimentation showed that the chyle contained a ferment capable of destroying the sugar in the blood and that this agent originated in the pancreas (Lépine, 1890). In a following work, Lépine and Barral carried additional experiments using the blood or the chyle extracted from the thoracic channel of a dog (Lépine and Barral, 1890d). In these experiments, 10 g of blood were received into 40 g of an aqueous solution of pure glucose (0.5/1000) mixed with 1/1000 of thymol, and the mixture left in a stove for one hour at 41°C. The blood was titrated with Fehling's liquor before and after this operation. Experiments done with the blood of a healthy dog indicated a sugar loss of 4 to 6%, against zero loss for the blood of a dog deprived of its pancreas. A similar experiment carried with chyle indicated that the loss was 8 to 10%, that is, almost double that obtained if the blood had been mixed with the sweet solution. Everything being equal, normal blood abandoned to itself lost substantially more sugar than the blood of a dog made diabetic by the ablation of the pancreas. Within 10 minutes at 40°C, a diabetic blood lost, at the most, 8% of its sugar, against 38% for a normal blood. Lépine and Barral found that the temperature level was the most important factor affecting the destruction of the sugar: Three identical samples were left for one hour at temperatures of 51°, 41°, and 21°C; the sugar loses were 47%, 38% and 6% respectively. Asphyxiating a dog in confined space at 38°C, showed that during the asphyxia period the blood lost substantially less sugar: 21% before the asphyxia against 8% during the crisis. Similar results were obtained when the blood was circulated through a living tissue (kidney) (Lépine and Barral, 1890d, 1891fg, 1892a).

5. The saccharifying and glycolytic ferments

Barral wrote that the study of the different varieties of experimental diabetes, the mode of action of diverse medicines, and the resulting

organic modifications in animals, required the dosage of sugar and glycogen, as well as determination of the glycolytic and saccharifying powers of blood (defined as the percent loss of sugar experimented by blood maintained for one hour in a water bath at 38°–39°C) (Barral, 1890b; Lépine and Barral, 1890bc). He and Lépine had developed for this purpose an analytical method that was very fast; three hours were sufficient. It was based on sampling the blood in the artery of the animal by introduction of a glass cannula provided with a rubber tube; the first drops of blood were discarded and the following liquid cooled and analyzed as described before.

According to Barral, the destruction of sugar (glycolysis) was not the effect of micro-organisms, as shown by working the blood under strictly aseptic conditions (Barral, 1892). The existence of a glycolytic ferment was demonstrated by the following facts, many common to the known ferments: (a) Glycolysis did not take place with blood that had been heated at 54°C, at this temperature the ferment was totally destroyed; (b) the glycolysis was almost nil at 0°C, very weak at 10°C, and increased with temperature up to a maximum at about 50°–52°C; (c) the ferment was very soluble in distilled water and diluted glycerin, and insoluble in alcohol; and (d) centrifugation of the blood at low temperature, yielded a serum rich in glucose and albuminous substances and, generally, not containing the glycolytic ferment. The resulting globules, washed by centrifugation at low temperature with saline (1%) water, mixed again with saline (1%) water, left alone for one hour and then again centrifuged, yielded a liquid rich in albuminous substances but still able to destroy the sugar. This experiment proved that glycolysis should not be considered a vital property of blood albumen because a vital property could not be transported (Lépine and Barral 1891a; Barral, 1892).

According to Barral, the following facts and experiments proved that the ferment was located in the white globules: (a) although the chyle that did not contain red globules, it lost its sugar under the conditions that determined the glycolysis of blood; (b) the jugular of a dog, swollen with blood, was suspended in an enclosure at low temperature. After the globules had separated, the jugular was divided into three segments by means of two ligatures separated by several centimeters, one above and the other below the surface of separation. Glycolysis was completely absent in the plasma while it was weak or nil in the layer of red globules and very intense in the middle section containing the white globules, plasma, and red globules. Since the two last layers did not contain ferment, it necessarily had to be present in the white globules; (c) the serum of centrifuged blood seldom possessed glycolytic power. Separation of the different layers of globule with a syphon showed that the ones having the largest number of white globules had the greatest glycolytic power. Normally, the upper layer contained the most of white globules and showed the highest glycolytic power; and (d) the fibrin obtained by beating cold blood strongly compressed into a lump, contained a large amount of white globules, as shown by microscopic examination. Addition to a solution of glucose resulted in a decrease of the sugar present. This glycolysis was not due to the fibrin: maceration with water for several minutes showed that only the liquid had glycolytic power (Barral, 1892).

The fact that the glycolytic ferment was located in the white globules did not allow finding it in the urine because it was not present in the serum. The opposite was true with the saccharifying ferment, it was present in the serum and hence in the urine. Glycolysis seemed to be produced in the blood by imbibition of serum in the white globules.

What was the origin of the glycolytic ferment? It was known that the blood of a normal dog lost more sugar than that of a dog made diabetic by ablation of the pancreas. Hence, normal blood had

something that was not present in the blood of a diabetic dog. This something was the glycolytic ferment located in the white globules. It also proved that it originated, a least partially, in the pancreas but *did not accumulate*. In addition, experimental evidence indicated that the causes that produced the vasodilation section of the pancreas of the vessels resulted in an increase of the glycolytic power (Lépine and Barral, 1891m). According to Barral, the following facts proved that the ferment was present in the blood circulating in the vessels: (1) blood kept in the jugular of a dog lost its sugar when mixed with blood returning to the jugular; glycolysis of a blood that did not contain glycogen was larger during the first quarter of an hour than in the following quarters, indicating that the ferment *pre-existed* and was not formed little by little; (2) circulating defibrinated blood through the kidney of a dog by means of the Jacobj pulsatile pumping mechanism (Jacobj, 1890) showed that the muscles retained their irritability while the leaving blood was black and reentered the arteries totally red, even hours after the end of the experience; (3) blood kept for 24 to 48 hours at 0°C had a weaker glycolytic power than that it had after defibrination; (4) the serum obtained by centrifugation of defibrinated cold blood generally did not contain glycolytic ferment. The ferment was not formed, little by little, in the serum after the exit of the blood from the vessels; and (5) transferring blood from the artery of a normal dog to the vein of a dog made diabetic by ablation of the pancreas, resulted in a notable decrease of its glycosuria (Lépine and Barral, 1891m).

Lépine and Barral determined the values of the glycolytic power of human beings suffering of different illnesses (Lépine and Barral, 1891d) (Table 1). They inferred, from the values obtained and the experiments with dogs, that the glycolytic power of a healthy man was well above 25 (Lépine and Barral, 1891d).

Table 1. Sugar content and Glycolic power for different Illnesses

Illness	Sugar g/1000 g of blood		Glycolytic power (% loss)
	Immed-iately	After 1 h at 39°C	
Pneumonia	1.20	0.78	35
Pneumonia	1.04	0.78	25
Uremia	1.10	0.77	23
Obesity	1.17	0.89	24
Diabetes	5.07	4.9	3.5
Diabetes	4.54	4.47	1.6
Diabetes	3.48	3.23	7.0
Diabetes	2.17	2.05	5.5
Diabetes	3.38	3.3	2.1

6. Pathology of Diabetes

Lépine and Barral wrote (Lépine and Barral, 1891a) that understanding the pathology of diabetes required taking into consideration the following facts: (1) the blood of a healthy dog circulating during several hours through the kidney (isolated from the body) lost more sugar than that of a dog deprived of its pancreas. It was clear then that normal blood had something additional than the blood of a dog deprived of its pancreas; (2) this something was a soluble ferment that water could extract from the white globules and that was destroyed at 53°C, while the saccharifying ferment existed equally in normal blood and kept its full activity at the above temperature; (3) glycolysis of arterial blood was stronger in the healthy dog than in the pancreatic one. It was even more advantageous if the ligature of Wirsung's duct (joining the pancreas to the common bile duct) was practiced on the latter animal. This fact constituted an additional proof that the glycolytic ferment did not originate from the pancreatic juice pouring into the intestine; (4) glycolysis of the blood of the pancreatic veins of a healthy dog was substantially more energetic than that of the splenic vein (draining blood from the stomach fundus and part of the pancreas), or any other vessel; (5) the authors always found that

the glycolysis of a diabetic man decreased in absolute value, a remarkable fact considering that at equal circumstances glycolysis became stronger when the amount of sugar increased (Lépine and Barral, 1891a).

In a following paper Lépine and Barral reported their results on the *in vitro* destruction of the sugar in blood (Lépine and Barral, 1891b). For this purpose, 250 cm^3 of blood extracted from a healthy dog were defibrinated, filtered, and then divided into five equal portions. Before titration, the blood of the first portion was mixed with sodium sulfate at 80°C in order to destroy immediately the glycolytic principle. Three other portions were submerged separately, and for one hour, in three water baths held at 39°, 46°, and 52.5°C, respectively. The remaining portion was poured, dropwise, into a flask held in a water bath at 54°–52.5°C, and kept there for one hour. After one hour, all the portions were analyzed for sugar content. The results indicated that the amounts of sugar in the flasks at 39°, 46°, and 52.5°C contained sugar in an amount increasingly smaller than in the first flask, and the flask at 52.5°C contained sugar in an amount equal to that of the first portion. These results justified the statement given previously that the glycolytic ferment become more active as the temperature increased until about 54°C when its activity ceased suddenly. At that temperature the blood kept its fluidity, but had blackish tint, due to the production of methemoglobin, recognizable under the microscope. Most of the red globules had been destroyed (Lépine and Barral, 1891b).

All things kept equal, the defibrinated blood of a dog, kept for one hour at 39°C, lost more sugar in winter than in summer. This meant that the ferment was more active in winter. The defibrinated blood of the portal vein of a dog in digestion, kept for one hour at 39°C, lost more sugar than the blood of the splenic vein and the arterial blood of the same dog. This result showed that the ferment left the pancreas not only through the lymphatic of the organ but also, and more largely, by the venous reticules of the pancreas (Lépine and Barral, 1891b).

7. Effect of Living Tissue

Lépine and Barral also studied the glycolysis of blood circulating through a living tissue (Lépine and Barral, 1890b, 1891f). For this purpose they completely bled a dog and defibrinated the blood in a cold flask. One of the dog's kidneys was removed, immersed in water at 39 °C and then a given volume of blood, 500 cm^3 for example, was circulated by means of the apparatus of Jacobj. The properties of the tissues and of the blood were kept as good as possible. Under these conditions the muscles kept their irritability. The blood leaving the organ was black; the oxygenation provided by the apparatus turned it completely red before it reentered the artery. A sample was taken after every circulation through the whole apparatus. Analysis of the blood showed that its flow through a live tissue isolated from the nervous system resulted in a gradual decrease of the sugar content (about 60% within the first hour). Repeating the experiment with a dog rendered diabetic by removal of the pancreas indicated that the sugar loss was only 30% during the first hour. Lépine and Barral believed that this procedure was more exact than the corresponding study made *in vitro* (Lépine and Barral, 1890b, 1891g).

8. Effect of Medicines

Another paper described the effect of several medicines [antipyrine, jambul (Java plum), morphine, and phloridizine] on the glycolytic ferment (Lépine and Barral, 1892c): (1) antipyrine was found to diminish the action of the ferment, both *in vitro* and *in vivo*; (2) treatment of a dog with a mixture of one gram of the fluid extract of jambul (*Syzgium cumini*) per kg, showed that the glycolytic power was clearly increased, justifying the employment of this fruit in the treatment of diabetes; (3) a strong dose of morphine increased significantly the glycolytic power, accompanied

by production of glycosuria, which seemed to be connected with the increase in the saccharifying power of blood. Lépine and Barral believed that more experiments were needed before deciding the possible use of this alkaloid in the treatment of diabetes; and (4) it was known that ingestion by a dog or about 0.5 g of phloridizine per kilo made it rapidly diabetic. Lépine and Barral studied in detail the changes in the glycolytic and saccharifying powers of blood, three hours after ingestion of phloridizine, and found that both were increased. In addition, after the following hours, there was an increase in the saccharifying power of the urine. In other words, the phloridizine-induced diabetes did not diminish glycolysis but led to an exaggeration of the production of sugar (Lépine and Barral, 1892c).

The discovery of the glycolytic ferment was, undoubtedly, the most important scientific contribution of Barral and Lépine. Their systematic work on the subject, accompanied by the execution of a large number of carefully designed experiments, demonstrated that this agent was responsible for maintaining the sugar level in the blood, its origin in the pancreas, and its direct relation to diabetes. In the early 1920, the Canadian and Scottish scientists Fred Banting (1891–1941), Charley Herbert Best (1899–1978), John James Rickard Macleod (1876–1935), and James Bertram Collip (1892–1965), demonstrated that this ferment was insulin, a peptide hormone that regulated the metabolism of carbohydrates, fats and proteins. In 1923 Banting and Macleod were awarded the Nobel Prize in Physiology or Medicine for their work on insulin.

Appendix 1

Victor Barral

Étienne Victor Barral (1860–1938) was born in Saint-Foy-lès-Lyon on August 12, 1860, the son of Charles Luc Barral and Madeleine Gontelle. He took his basic education at the Lycées Saint-Rambert and Ampère in Lyon. After graduation he entered the Faculté des Sciences and received his degree of licencié ès sciences in 1884, of pharmacien de 1er classe in 1889, his doctorate in medicine in 1890, after successfully defending a thesis on sugar in blood and a new theory on the glycolytic ferment (Barral, 1890a), and his doctorate in physical sciences in 1895 from the faculty of physical Science of Paris, after defending a thesis about the synthesis of polychlorinated derivatives of phenol and benzene (Barral, 1895b). He occupied several academic positions: extern of hospitals (earned by competition in 1886), préparateur for the chair of hygiene and bacteriology held by Adrian Loir (1862–1941) at the Faculty of Sciences in Lyon, préparateur for the chair of organic chemistry and toxicology (1884–1888) held by Paul Cazeneuve (1852–1934) at the Faculty of Medicine in Lyon, head of medical works (1888–1892) at the medical clinic of Raphaël Lépine (1840–1919) at the Faculty of Medicine in Lyon, head of works and adjunct (1892–1895) for the medical and pharmaceutical chemistry chair held by Louis Hugounenq (1860–1942), and Professor at the Société d'Enseignement Professionnel of Rhône (1887–1891) (Barral, 1910; Desgrez, 1992; Anonymous, 2018a). His work in analytical chemistry and in the chemistry of polychlorinated derivatives of phenol led to appointments as commercial tester at the Lyon Guarantee Bureau (1908) and membership in the Rhône's Departmental Board of Hygiene. During the First World War he served as first-class medical officer, as head of the laboratory of expertise of the Desgenettes Military Hospital, and worked on the development of protection measures against poisonous gases. Barral was corresponding member of the Académie de Médicine (1938), member of the Association Française pour l'Avancement des Sciences, member of the Société des Sciences Médicales de Lyon, of the Société Médicale des Hôpitaux de Lyon, of the Société de Sciences Naturelles de Tarare, of the Société de Pharmacie de Lyon, and of the Société Chimique de Paris. In 1908, on occasion of its 50th anniversary, the latter appointed Barral Chevalier du Mérite Agricole. In 1898 he was elected Officier de l'Académie and in 1905 Officier de l'Instruction Publique (national French orders for distinguished academics). In 1917 he was appointed Officier of the Légion d'Honneur. Barral died on March 24, 1938.

Barral, alone or with Raphaël Lépine, wrote about 160 papers and books on the subjects of inorganic, organic, and biochemistry, physiology, diabetes, mineralogy, etc. As customary for candidates to the Académie de Médicine, he published a booklet describing the results of his scientific research (Barral, 1910). In addition to the subject described above he studied the poisonous properties of swamp star anise (*Illicium parviflorum*) (Barral, 1889); hexachlorophenol (used as a fungicide) and other polychloro derivatives, their reactions and properties (Barral, 1893, 1894ab, 1895ab); the chlorination of phenyl carbonate (Barral, 1899) the preparation of mixed carbonic ethers of

phenols and alcohols (Barral, 1900); the crystallization of arginine picrate (Barral, 1907a); the transformation of potassium bitartrate into potassium bicarbonate by means of molds (Barral, 1907b); colored reactions for several medicines (phenoacetin, pyramidon, cryogenine, abrastol, hermophenyl, pilocarpine, acetanilide, chloral, and hydroquinone) (Barral, 1907c, 1910), etc.

Appendix 2

Raphaël Lépine

Jacques Raphaël Lépine was born in Lyon on July 6, 1840, the youngest of the two sons of Jean Baptiste Lépine, an accountant, and Joachime Emmanuelle Vegerano. His brother Louis Jean Baptiste (1846–1933) became Préfet de Police of the Seine. Raphaël Lépine married Mathilde Koechlin; one son was born of this union (Jean, 1876–1969). After finishing his basic education he enrolled as student in the Faculty of Medicine of Paris from where he received his degrees in 1870 and 1875 after successfully defending two theses, one about pneumonic hemiplegia (Lépine, 1870), the other about location in brain diseases (Lépine, 1875). He served as intern in the hospitals in Lyon (1860) and Paris (1865), préparateur of the course on comparative and experimental pathology and head of the clinic of the Faculty of Medicine in Paris. In 1872 he was appointed physician at the Central Bureau of the Parisian hospitals and adjunct at the Faculté de Médicine de Paris, after winning the pertinent aggregation competition (the door to an academic career) with a thesis about caseous pneumonia (Lépine, 1872). Eventually he became Professor at the Faculty of Medicine at Lyon. As customary for candidates to the Académie de Médicine (Section Médicine et Chirurgie), he published a booklet describing the results of his scientific research (Lépine, 1887). He began his research activities studying the brain, the nervous system, and illnesses of the spinal cord, using the caves of the hospital as his laboratory. He individualized the pseudo-vulgaris paralysis, where the patient presents set of bilateral motor disorders (occurring on both sides of the body), and psychic disorders. He was the first to recognize the pancreas as a true endocrine gland, which regulated the action of glucose. His work and that of his students set the road to the discovery of insulin. He identified renal diabetes, an illness where glucose is excreted in the urine, even when the sugar level in blood is normal or below normal.

Lépine, alone or with others, wrote about 120 papers and books on different aspects of medicine (physiology, diabetes, nervous system, hematology, toxicology, infections, nutrition, etc.) (For example: Lépine, 1895; Lépine and Aubert, 1885; Lépine and Lyonnet, 1897; Lépine and Roux, 1885). He was member, secretary, and president of the Société de Biologie, and member of the Société Anatomique and of the Société des Sciences Médicales de Lyon. Lépine died on November 17, 1919, in Menton (Alpes-Maritimes).

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