THE PUBLISHING HOUSE
OF THE ROMANIAN ACADEMY



# UNEXPECTED ENIGMAS OF THE SPLEEN FROM THE BOOK: "THE STRUCTURE OF THE SPLEEN" BY N.C. PAULESCU

Constantin IONESCU-TÎRGOVIŞTE

"N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania.
*Corresponding author:* Constantin Ionescu-Tirgoviste, E-mail: cit@paulescu.ro

*Accepted January March 6, 2020*

One of the main organs of the human body is the spleen. For this reason we publish in here Paulescu's first thesis obtained in the Medicine University of Paris in 1897, under the coordination of E. Lancereaux.

## INTRODUCTION

Spleen is by excellence a vascular organ. Its functions are related to its role as a reservoir of blood (~ 150 ml of blood may be released into the systemic circulation under hypoxia conditions), but also to filter the blood entering the spleen and to "clean" it of the used erythrocytes, of some encapsulated microbes entering the systemic circulation and triggering an immune defence and surveillance reaction against them. It also produces and stores a large number of platelets.

The architecture of the organ as a whole is adapted to this function. Unlike other "solid" organs (liver, kidney), the outer shell of the spleen consists of an elastic fibro conjunctive tissue that allows it to rapidly change in volume. This can be doubled quite quickly, as under unexpected effort, when the contraction of the smooth muscle fibers existing in the capsule and in the intrasplenic trabeculae, a quantity of blood is expelled from the spleen before going through the complete filtration path, when its volume decreases. In this case, the filtering process will be done the next time the blood returns to the spleen in its uninterrupted flow.

In a section of the spleen, seen with the naked eye or a magnifier, one can see a red-emitting mass (red pulp), inside of which there are small, very bright areas (the white pulp Of the spleen), as well as the carriages that leave the inside of the spleen capsule inwards. These are more noticeable in splenomegaly. Such sequela (the persistence of an enlarged spleen) was often encountered in our country, in the southern counties along the Danube, areas with adjacent lakes full of mosquitoes. At anamnesis, we find out that in childhood people have had "colds" (malaria).

The interest in the spleen was awakened by the discovery of the PhD thesis supported by our illustrious physiologist Nicolae Paulescu in 1897 and published in Paris the same year[1]. I was surprised to note that Paulescu's interest in the study of this organ was due to the uncertainty surrounding the structure and functions of this organ. Whoever reads this thesis remains surprised by the many studies dedicated the spleen in the 19$^{th}$ century, which our young researcher has analyzed in a comprehensive, critical and competent manner. He had the chance to witness the many clinical-necrotic encounters that his master Lancereaux did regularly. In this context, it is surprisingly the shadow cone in which this organ has remained almost a century. The introduction of laparoscopic videos into the current surgical practice has made this "forgotten" organ[2] to benefit from an increasing interest. After the cord, the spleen is the second parenchymatous organ whose volume will undergo such a large variation within 24 hours. According to the information from Paulescu's thesis, it results that the organ volume increases postprandial and decreases after a physical effort. On the other hand, the variability of the video-laparoscopically visible spleen vessels in the hill of this organ led to the idea that such variability might exist in the internal spleen structure. In such a situation, the "recourse to history" becomes mandatory because it can provide information from a time when the "time is money" slogan still does not exist and the curiosity and patience of the researchers was much higher.

## THE RECOURSE TO HISTORY

The spleen is known as an organ from ancient times, being mentioned by Aristotle in the 4th century BC. Connected closely with the pancreas through the spleno-pancreatic ligament, explains why among the 12 acupuncture meridians described by the Chinese about 3,000 years ago, one of them is called the spleen-pancreas, it starts from the inner angle of the thumb, goes upward on the inner face of the shank and the thigh, then on the latero-abdominal line, ending in the axillary region (Fig. 1). The indications of the points of this meridian are numerous, although these do not have to be interpreted by the Western knowledge of anatomy and physiology. Their choice is based on some interesting philosophical-physiological concepts, based on a detailed anamnesis and on careful skin observation, the appearance of the tongue, the prolonged palpation of the pulse, etc. As the oldest method of treatment that is practiced today as it has been 3000 years ago, I have always considered it worthwhile being re-evaluated and reinterpreted.

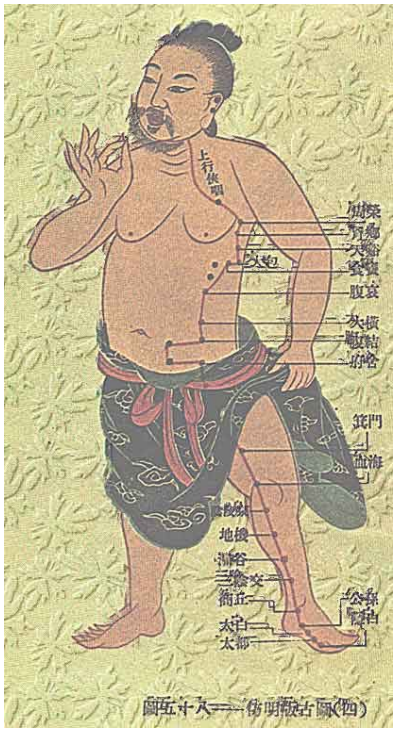

Figure 1. The spleen-pancreas meridian route.

In Europe, Marcello Malpighi (1628–1694) is perhaps the first scientist who try to break the galenical anatomical dogmas, which was established by the Greek physician Galenus (130–260 AD) after his establishment in Rome. Encyclopaedic spirit, Galenus had played a major role in synthesizing the knowledge of his time, succeeding in transforming his writings into a millennial dogma, being spread throughout the Roman Empire and beyond.

The Medieval Dogmatism, reached the age of the microscope, revolutionizing the natural sciences in general, and the medical ones in particular. Malpighi had a rudimentary microscope that grew between 100–150 times, far less than the lens of the "lenses", Antony van Leeuwenhoek – 1632–1723, much better. The use of this microscope with great curiosity and passion also allowed him to describe numerous structures in the vegetable and animal world, and had the chance to live 91 years, which was unusual at that time. In turn, young Malpighi, an anatomy professor at the age of 38 in Bologna, is putting in motion the new microscope-objectified data that no longer corresponded to Galenus's doctrine, which has attracted many inconveniences from his colleagues, suffering physical reprisals and burning his house and his laboratory. That's why he had fled for a while when in Pisa, when in Rome, to get rid of gangs paid to terrorize him. However, he is the first to discover and describe blood capillaries (the important link that was missing in the description of blood circulation by William Harvey – 1578–1657).

Malpighi intuitively understood the lobular organization of many organs: liver, kidney – where he described in some papers[3,4,5,6] the kidney glomeruli (which bears his name), the lungs or the lymphatic follicles in the spleen (later referred to as the Malpighi corpuscles). He also described the "tactile organs" in skin, later known as Krause cells[7], but also the sweat glands and the sebaceous glands of this organ.

Figure 2. Macello Malpighi 1628–1694.

From the famous painter Albreht Dürer (1471–1528) we have a self-portrait in which, with his right hand, he indicates the region of the left hypochondrium, where the spleen is marked by a circle. He had suffered, maybe malaria or other benign affection, taking into account that he lived 57 years.

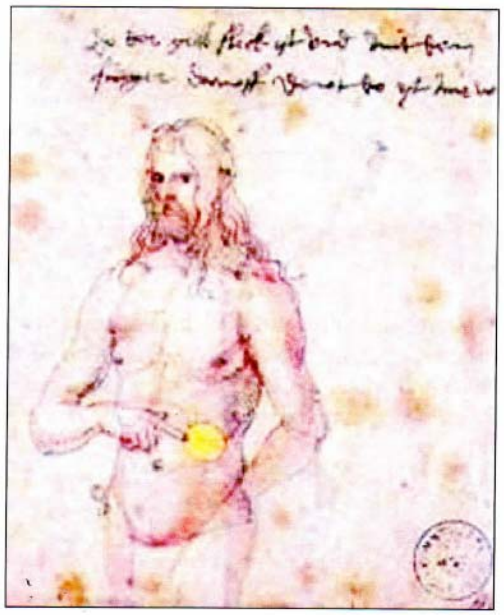

Figure 3. Albrecht Dürer (1471–1528) indicating his painful spleen area.

At the end of the nineteenth century interest in the spleen study increased greatly. The data published in this period being contradictory, and the spleen functions quite obscure despite the fact that the largest splenomegaly were met in malaria that haunted many warmer regions in southern Europe, around larger rivers or lakes, or in liver cirrhosis, which in France was largely due to the high consumption of alcoholic beverages. After the French surgeon Joule Emile Péan (1830–1898) performed the first splenectomy in 1867, others followed, observing that the removal of the spleen at an adult age (especially after abdominal trauma) was not followed by visible inconveniences. In addition, the necropsy discovery of a large spleen, without the patient having a notable pain, led to the idea that the spleen is a non-useable (dispensable) organ.

In 1897, when Nicolae Paulescu supported his doctoral thesis "***La structure de la rate***", published in the same year at Doin Publishing House in Paris, the accumulated data was so contradictory that, as he mentions, he felt the need to throw a New light on this obscure field of medicine. A well-known nature scientist in general, and finding that the spleen is an organ present in all living creatures (amphibians, fish, birds, mammals), he knew that such an organ can't be meaningless. After a pertinent review of histological data published so far, based on the necrophetic analysis of splines obtained accidentally from seemingly healthy individuals, but also from a long-observation of a patient with a large malaria splenomegaly, he concluded that the complicated structure of the spleen (presented and interpreted differently by the most important time histologists) consists of end-functional units similar to the small lobes in the lungs and, to some extent, to the liver. To the same conclusion came after more than 130 years the researchers who benefited from advances abdominal imaging, electronic microscopy, including the three-dimensional reconstruction of the lymphatic follicles (Malpighi corpuscles, which make up the "white pulp"), which is the most specific spleen structure along with the venous sinuses ("Billroth columns") that make up the "red pulp".

## THE SPLEEN IN THE NEW MILLENNIUM

Stimulation of interest for the spleen study has occurred over the past two decades after the introduction of laparoscopic interventions and the possibility of performing partial splenectomy[8]. It should be noted that these techniques have been used preferentially in children suffering from haematological disorders: red cell spherocytosis and thrombocytopenia. The excellent monography of Professor Cătălin Vasilescu published in 2016 and then the synthesis published in "Actualities in Internal Medicine" published in 2017[8] revealed many morpho-functional features of this organ, some of them unique and still incompletely clarified. At first glance, taking into account the advances in imaging technology, including three-dimensional reconstruction of intrasplenic structures in the two major areas of the spleen ("white pulp" and "red pulp"), has amplified the interest in understanding the precise blood flow of this two large anatomic-functional areas, still in the hypothesis field.

If the images of three-dimensional reconstituted structures[9] are spectacular, blood circulation in the lymph follicles of the "white pulp" and its passage into the venous labyrinth of the "red pulp" still has many unknowns. Anatomy studies have succeeded in tracking the arteries and veins which, after penetrating through the splenic hill and dividing successively on several occasions, being included

in the trabeculae of the intrasplenic septa, can be traced by delimiting the "segments" (the lobes) that have terminal circulation and can be removed surgically (segmental splenectomies), which subsequently subdivide into lobules (structures smaller than the lobes, but which may also have a terminal circulation). The data are uncertain, as in vivo study could not tell the circulatory dynamics inside them.

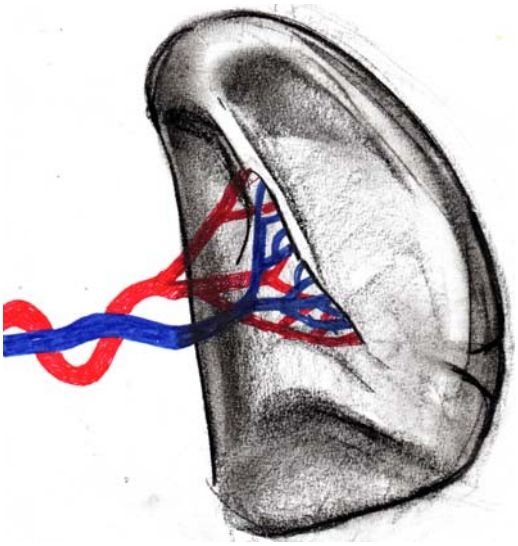

Figure 4. A common variant of large extra spleen vessels.

A classic image of the splenic artery and the first divisions occurring in the organ hill is shown in Figure 4. As you can see, after the first 3 divisions there are smaller ones that penetrate inside the spleen, here in number of 7. This is only one variant, as there are cases in which only the first 3 divisions are in the splenic hill, the other are dividing within the spleen and obviously can't be seen by the surgeon to perform an intervention on this organ. In Vasilescu's earlier work[8], there are numerous variants of extra-arterial blood and venous circulation, which the surgeon must always take into account. Their presence suggests a large heterogeneity in the overall spleen structure.

What we know is that after many subdivisions of the arteries and veins, at some point, they come out of the trabecular envelope, at which point the arterioles break out of the veins, following a path around which a lymphatic region is formed which comprises one or more follicles associated with a large number of arterial capillaries, which are branching like "pleated loops" and which have been termed "penicillary arteries" (from the Latin "penicilum", which means brush) and form a capillary network, an area called the "marginal zone of the lymphatic follicles". It delimits the lymphatic territory, within one or more lymphatic follicles can appear around some capillaries. Some of them are rich in T lymphocytes and others are rich in lymphocytes B. In Fig. 5, Vasilescu redesigns the vascular architecture of the white pulp inspired by a three-dimensional reconstruction made by Kuzumi *et al.* in 2015[9]. Such reconstructions have a certain degree of relativity, due to the large inter-individual heterogeneity of the intrasplenic microcirculation and, possibly, the area from which the analyzed splenic tissue is taken. Obviously, these images can only suggest the dynamics of processes taking place in the spleen, which depend on many factors, both physiological and pathological.

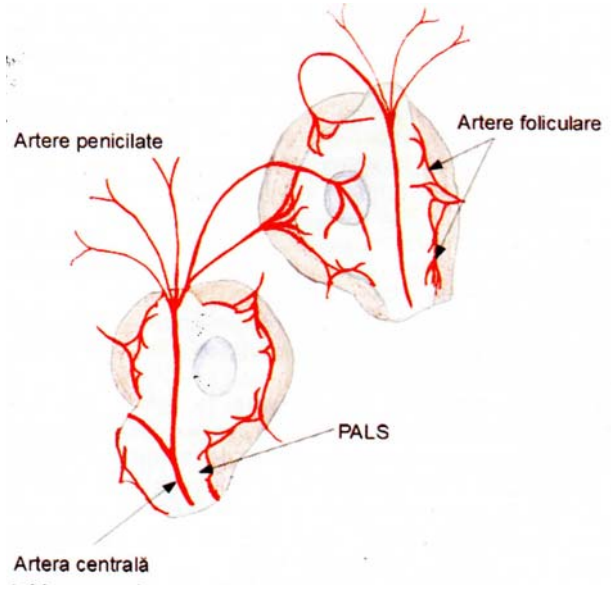


Figure 5. Microvascular architecture of white pulpe in humans.

This vasculo-lymphoid assembly contributes to blood filtration for all antigens (viruses, microbes, parasites) that have reached the spleen and will react to them in an appropriate manner.

From the marginal area of these "immune territories", the blood will pass into the red pulp characterized by the existence of spleen-specific venous structures called sinuses or venous defects (Paulescu's calls it a "huge bloodbath"). Inside these sinuses Billroth described the cellular columns that bears his name. Inside, erythrocytes are subject to "quality control". The young, elastic and normally conformed erythrocytes will be released into the lacunar venous system by being directed to the roots of vein capillaries. Then will be directed to the place where the vessels have detached from the arterioles, following the reverse circular path to the arterial tract, Forming larger

veins until their merging into the splenic vein that occurs at the level of the hill joining the corresponding splenic artery. The inside of the spleen is spongy so that tracking the bloodstream through such a labyrinthine system is virtually impossible to follow. We do not yet know what driving force directs this flow which at least theoretically must follow a pressure gradient. Should muscle fibers dispersed within the spleen tracts play a role in this?

## THE ANATOMIC-FUNCTIONAL UNIQUENESS OF THE SPLEEN IN PAULESCU'S VISION

After reading the text of Paulescu's thesis, as well as the completions made in the physiological treaty published in 1920[11], there are some interesting ideas, some of which were confirmed and others could be resumed to be confirmed or denied.

It is believed that most of the blood entering the spleen (~ 90%) passes into the "large spleen blood pool"[1], located in the "red pulp" of the organ. Before reaching these lakes, the lobar arteries branched into several small lobular arteries, each having a specific area of distribution, until at the extremity of the intrasplenic arteries the arteries descend as a vein tract, delimiting between them the "functional final units" described by Paulescu[11]. Much has been said about the "closed" or "open" system of blood circulation in the spleen. It is obvious that the blood entering the arteries, arterioles and blood capillaries will eventually return to the venules, veins and the large splenic vein. Inside the spleen, however, the blood will have to pass through both the "white pulp" to perform its immune functions and the "red pulp" to perform its filtering function.

It is interesting to note that in the "red pulp" an extensive network of reticulin fibres and intercellular matrix has been identified, which interconnects the structures and explains the "spongy" aspect of the labyrinth sinus, in which the Billroth columns are found, and where the identification of the arterial and the venous component, is problematic. In this region, there are "splenocytes", which are isolated cells in the spleen, after removal of erythrocytes. Their specificity is still incompletely defined, yet another reason for a closer study of this organ.

Depending on the "perceived" needs of the circulating antigens detection system present in the blood, the number of lymphatic follicles may be higher or lower. Both with regard to the way blood circulates in the splenic capillaries, as well as their structure and function, there are several points of view. Each can be sustained, the differences between them being given by the dynamics of rapid adaptation of splenic structure and function under different conditions. In this way, some will surprise a certain moment, and others will surprise another moment, even if the investigation takes place in the same person but in two different days. Obviously, the differences may be much greater when looking at images obtained from different individuals, and obviously those in which the spleen is subject to unusual demands, such as hereditary spherocytosis or thrombocytosis, to name only those two more frequent haematological disorders in paediatric medical practice, accompanied by splenomegaly.

Between the endothelial cells forming Billroth trajectories there are spaces of precise size whereby the erythrocytes entering these conduits have to pass out of them into the drained bloodstream then to the venous system. If the shape (spherical or malformed) or the erythrocyte elasticity is low and can't pass through these strains, they will be swallowed by the numerous macrophages existing within those channels where iron and globin are recovered and sent for recycling. Then other erythrocytes will form in spleen or in the marrow and the rest of the erythrocyte shell will be proteolyzed and finally removed from the system.

In Paulescu's Ph.D. thesis there is information, which I have not found in other publications, namely that at one point the arterial capillaries grow relatively suddenly from a diameter of about 10–15 μm to 35 μm, making the necessary spaces for the formation of Billroth trajectories. This represents the canalicular system in which erythrocyte filtration takes place and, possibly, the retention of some cellular debris present in the systemic circulation. They will be embedded in large numbers of macrophages inside these channels. We note that a normal erythrocyte has a good elasticity and a diameter of 7.5 μm and a thickness of 2 μm, much smaller than the inside of the Billroth channels.

Interestingly noteworthy is that a small percentage (~ 10%) of the blood of the spleen capillaries passes directly into the venous capillaries, shortening the vascular maze in the red spleen pulp. It is the expression of a "biological precaution" for situations where the splenic labyrinth should be avoided,

especially in pathological conditions, when the red pulp is mostly destroyed (in malaria, for example) when the anastomoses of the arteriovenous capillaries become evident. Paulescu makes an excellent description of this pathological situation (shown in Figure 6 after histological analysis of the spleen of a deceased patient following a long evolution of malaria), reaching conclusions that are difficult to see by analyzing only the normal splines. Fibrotic tissue results from damage to the extracellular matrix rich in collagen fibres, which lose their elastic characteristics, becoming hypertrophic and rigid. They encompass their scar structure and intrasplenic septa.

## THE SPLEEN: AN ORGAN WITH SPECIFIC FUNCTIONAL AND STRUCTURAL FEATURES

In order to achieve a higher filtration surface, the biological solution in the spleen was to produce numerous "filter ponds" that form the "red pulp" of this organ. This function is performed during blood passing through the "Billroth columns" mentioned earlier.

Inside the "red pulp" the number of Billroth columns is very large, they belong to the arteriovenous system, the endothelial cells having an elongated shape being arranged in parallel forming a kind of pipes similar to "barrels" open at both ends and fixed by means of collagen circles that maintain consistency and shape. Within these "barrels", there are numerous macrophages that will take up old or abnormal erythrocytes, recovering iron and globin, and processing the remnants of red cells or platelets removed from the system. In Figure 7 we present a suggestive image for these structures rendered by Paulescu[1], showing in Figure 8 a theoretical construction of how the erythrocyte control system works in a modern vision[10].

This sinusoidal arrangement produces a large contact surface between the blood entering the splenic artery and which must be filtered at least once in 24 hours, which would be impossible to achieve in a limited structure such as spleen (about 250 $cm^3$). If one were to calculate the total surface of these "filter ponds" it might be surprising to find values in the order of many tens of square meters.

"Billroth columns" are made up of elongated endothelial cells, forming a kind of "barrels" opened at both ends, so the blood is routed through its interior. Between two Billroth columns, there is a free space of 0.5–2.5 μm, through which normal erythrocytes pass into the venous sinuses and from there into the venules that successively enter the small intrasplenic trabecula, then in the medium and large (when they come back along with the arterioles) arriving in the larger vessels near the hill where, together with the innervation of the arteries, they are found in the vasculo-nervous pack that stretches between the artery and celiac vein and the spleen hill.

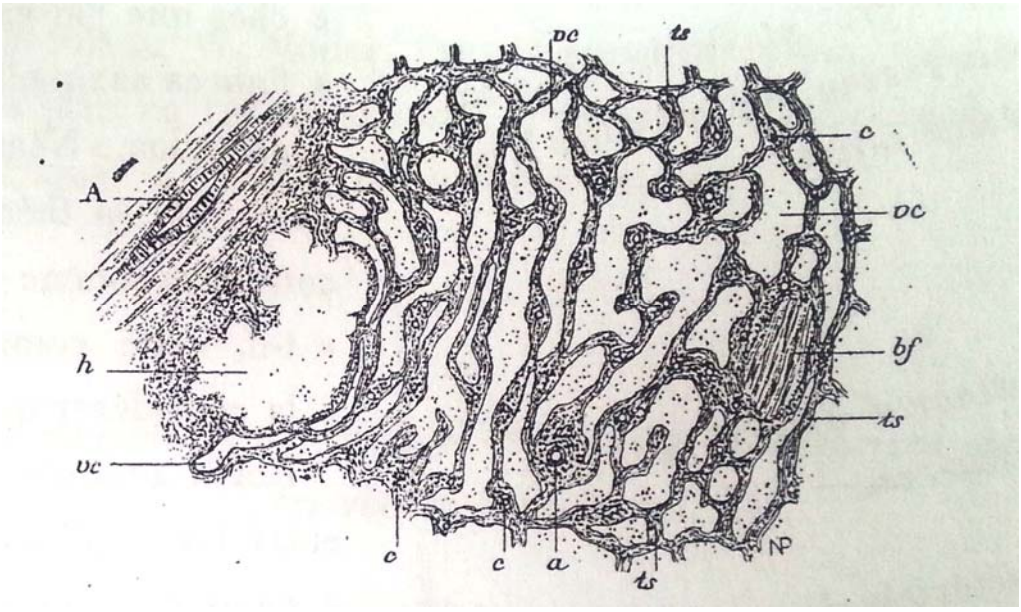


Figure 6. Cross-section of the spleen of a patient deceased during a recurring malaria attack. A – splenic artery; bf – interlobular fibrous bands; h – minor capillary haemorrhage; a – splenic arteriola; c – arterial capillary; ts – splenic trabeculae ; vc – dilated capillary vein.

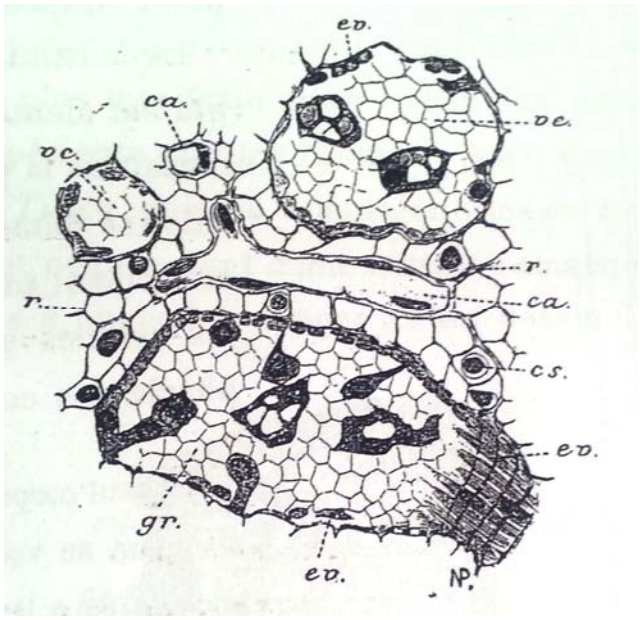


Figure 7. The Billroth Channels in Paulescu's view, bottom right corner.[1]

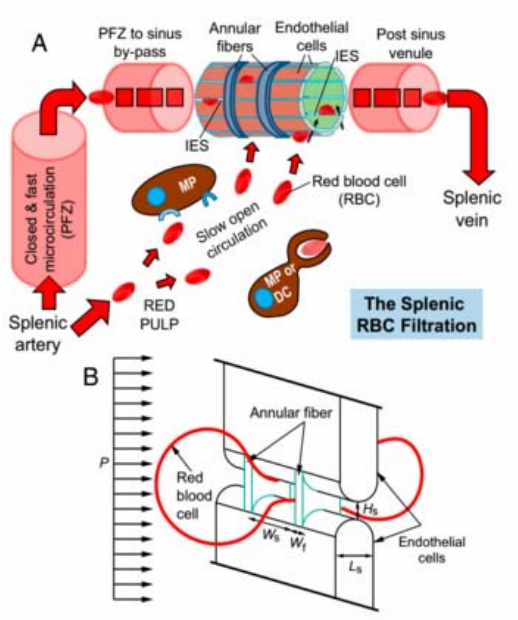


Figure 8. The same channels in Pivkin theoretical reconstruction of.[10]

The spleen filtering function, which reproduces in an ingenious manner the "decanting water ponds to become drinkable" and which sums up many square kilometres, is carried out within 150–200 grams of spleen tissue, made up of numerous "functional units" (1), each representing a small tailings pond, working in parallel with the other hundreds or thousands of ponds. Paulescu's analogy with pulmonary or hepatic lobes is an evidence of a good understanding of the anatomical organization of this organs, where the same major function takes place, but each one has a particularity in relation to the material to be "filtered". In the lungs $O_2$ capture and $CO_2$ and $H_2O$ elimination, in the liver processing the nutritional principles that come from the intestine through the portal vein pathway. In all these organs / systems, between the entering and exiting blood, there are physiological processes that can only be accomplished by multiplying the functional anatomical units that are repeated in all these organs.

Over time, there has been intense debate about the arterial or venous affection of small capillaries and arterioles. From Paulescu's analysis in 1897, the arterio-venous circulation in the spleen is finally complex and unique in its own way, hence the different interpretations offered by the researchers of his time. It is clear that both white pulp (Malpighi glomeruli, or lymphatic follicles) and red pulp are connected to a spleen artery.

The red pulp, the majority on the microscopic sections, due to its reddish colour, is the major headquarters of the "filtering" function of erythrocytes and possibly platelets. On this background, in place, there are small areas called white pulp that provide the immune filter function of this organ. These structures are included in a complex network of collagen fibres forming a complex matrix that explains the spongy character of this organ.

One of the extra information Paulescu brings is that the transition from the arterial capillaries to the "big bloodstream" to the vein is done when the diameter of the vessels suddenly passes from 10–15 μm to 30–35 μm forming the labyrinth system, including the Billroth columns. This labyrinth system can be considered to be the "root of the splenic venous system". However, the characteristic of the vascular endothelium and their basal membrane that is continuous, with no free zones between two endothelial cells, occurs only when the diameter of the venous capillaries reaches 70–110 μm. From this moment they enter the fibrous bands and from there through successively through thicker bands to the splenic hill. Only when they reach such a diameter joins the arterioles so that when they come out of the splenic hill, they will be part of the specific vasculo-nervous pack.

The lymph nodes emerging from the spleen have a separate route. According to Paulescu[1, 11], the lymph nodes appear in both the Malpighi corpuscles (the lymphatic follicles or the white pulp) and in the red pulp of the spleen. They are then identified in the intra- and interlobular fibro-intestinal tracts, then in the spleen capsule, coming out of the spleen at the level of the hill, from where

it goes to the peripancreatic nodes (especially the posterior ones), pointing to the Chyli tank, from where through the left thoracic channel it flows into the left sub clavicular vein.

The function of the spleen is not only to remove the non-conforming erythrocytes (culling function), it also contributes to the removal of the cell debris (pitting) from the blood, resulting from the destruction of some erythrocytes, with the possibility of regenerating a similar number of destroyed erythrocytes, either in spleen or (in adulthood) in the bone marrow, the major erythropoiesis post-puberty or even before it. This physiological function has to process more than 350 billion erythrocytes/day, ~5 million/second[12]. In addition, through the filtering process it is possible to identify the germs (those encapsulated which cannot be eliminated in the lymph nodes, but only here because of the spleen capacity to "opsonize" and destroy their protective cuticle) and parasites (Phalciparum plasmodium, the malaria agent), but also other circulating antigenic molecules or structures.

Taking these particularities into account, the spleen is one of the important components of the body's "defence system" (instinct), integrated into lymphatic structures, acting silently, continuously and effectively. Obviously, when its structure is abnormal or its functional capacity is overcome (as it happens in various red blood cell disorders), various severe conditions occur, some of which require splenectomy, either total (as previously) or only partial (as is currently recommended) with the development of laparoscopic techniques applied for the first time in this field by Cătălin Vasilescu in 2007[13]. However, there are many unknowns, both in the dynamics of intra-splenic circulation and the microscopic structure of the vasculo-cellular network with their individual functions. After analyzing the data published by Paulescu 130 years ago, we believe that we have a moral duty to respond to some of the hypotheses advanced by him then.

## WHAT ADDITIONAL INFORMATION DID WE INHERIT FROM PAULESCU?

1. Has brought solid arguments in favour of the major role of spleen in the body's economy.

2. Has advanced and described the "final anatomical-functional units" of the spleen found in the spleen lobules.

3. Has made a pertinent analogy of splenic lobules with similar repetitive structures identified in lungs, liver and possibly other organs.

4. He noted in the deep anatomical regions of the spleen in the "no land territory" found between arterioles and splenic venules, a region in which the blood vessel suddenly increases from a diameter of 10–15 μm to 30–35 μm. This change in the calibre of the vessels, after they have emerged from the vascular sheath together with the arterioles and venules, can explain how the blood vessels in the labyrinthine region are structured, where the Billroth columns are also found.

5. The hypothesis that, besides its known functions, the spleen may play a role in "nutritional assimilation", a term used by him to designate the major function of the pancreas, and contribute through his exocrine function to the digestion (containing carbohydrates, lipids and proteins) making the metabolites absorbable. Then, through the internal secretion, it produces a hormone capable of stimulating the use of three types of energy compounds in the peripheral tissues: carbohydrates, lipids and proteins. This vision was well expressed in 1912 in Traité de Médecine volume III, being experimentally demonstrated in 1916. Due to the interruption of activity of the Faculty of Medicine in 1916 following the occupation of the capital by the German troops by the entry of Romania into the First World War, data retained until that time was published in Volume 2 of the Traité de Physiologie Médicale. At the end of the eight-page chapter devoted to studying the effects of the pancreatic extract he has on carbohydrates and proteins, Paulescu concludes with the following visionary conclusion: "Our data sheds new light on the pathogenesis of this syndrome and provides the key to its treatment." The key to treatment was provided in the two major publications of 1921[14,15], representing the "birth certificate of insulin". This certificate is also reinforced by the patent entitled "Pancreas and its manufacturing process" under number 6254 of 10 April 1922, h 1402.

6. Paulescu's statement regarding the possible role of spleen in nutritional assimilation seems to be certified by some experimental animal studies[16] and prospective analysis of splenectomised patients in humans[17, 18]. If "splenocyte" produces a hormone involved in intermediate metabolisms, then we suggest that it be called "Splenine" as a lower sister of Pancreine.

7. Paulescu wrote the first Romanian monograph devoted to spleen in the nineteenth century, contributing by his observations to the knowledge of this still incompletely explored organ. I regret that we have not identified this major scientific creation, but I

am pleased that her republication comes in a more rapid time for this "forgotten" organ[2].

## BIBLIOGRAPHIC INDEX


1. Paulesco N. La structure du rate, Paris, et Doin, 1897.
2. Görg C. The forgotten organ: contrast enhanced sonography of the spleen. Eur J Radiol, 64(2):189-201, 2007.
3. Malpighi M. De liene in op. cit. Bologne, 1666.
4. Malpighi M. De pulmonibus epistola altere, Bologne, 1661.
5. Malpighi M. De viscerum structura, 1666.
6. Malpighi M. De renibus; in De viscerum structure ecercitatio anatomica, Bologne, 1666.
7. Malpighi M. De externo tactus organo anatomica observatio, Napoli, 1665.
8. Vasilescu C., Splina. De la laparoscopie la chirurgia robotică și înapoi. Editura Medicală, 2016.
9. Kusumi S., Koga D., Kanda T., et al. Three-dimensional reconstruction of serial sections for analysis of the microvasculature of the white pulp and the marginal zone in the human spleen. Biomedical Research, 36(3) 195-203, 2015.
10. Pivkin IV, Peng Z, Karniadakis GE. Biomechanics of red blood cells in human spleen and consequences for physiology and disease. Proceedings of the National Academy of Sciences of the United States of America, 7804-7809, 2016.
11. Paulescu N.C.: Traité de Physiologie Médicale, 3 vol., 2210 pag., Bucharest, 1919-1921.
12. Chadburn A. The spleen:anatomy and anatomic function. Semin Hematol. 37(1):13-21, 2000.
13. Vasilescu C., Stanciulea O., Arion C. Laparoscopic subtotal spenectomy in hereditary spherocytosis. Surg Endosc 21: 1678, 2007.
14. Paulescu N.C.: Action de l'extrait pancreatique injecte dans le sang chez un animal normal. Action de l'extrait pancreatique injecte dans le sang chez un animal diabetique.; Influence de la quantite de pancreas employee pour preparer l'extrait injecte dans le sang chez un animal diabetique Influence du laps de temps ecoule depuis l'injection intraveineuse de l'extrait pancreatique chez un animal diabetique; C.R. Soc. Biologie, No. 27, 23 julliet 1921.
15. Paulescu N.C.: Recherches sur le role du pancreas dans l'assimilation nutritive. Archives Internationales de Physiologie, tom 17, Fascicule I: 86-109, 31 Aôut 1921.
16. Yin D, Tao J, Lee DD, *et al.* Recovery of islet beta-cell function in streptozotocin-induced diabetic mice: An indirect role for the spleen. Diabetes 55:3256-3263, 2006.
17. Ley EJ, Singer MB, Clond MA, *et al.* Long-term effect of trauma splenectomy on blood glucose. Journal of Surgical Research.177:152-156, 2012.
18. Wu S, Fu C, Muo C, *et al.* Splenectomy in trauma patients is associated with an increased risk of postoperative type II diabetes: a nationwide population-based study. Am J Surg 208:811-816, 2014.