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The discovery of platelets and their function

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Summary

A brief history of the discovery of platelets is presented, starting from the first descriptions of "small blood corpuscles" to their identification as the third morphological element of blood, independent of red and white cells, by Bizzozzero, and the acknowledgment of their role in haemostasis and thrombosis.

The description of particles in blood smaller than leukocytes and erithrocytes is dated at the end of the eighteenth century. However, it was only between 1865 and 1877 that these corpuscles were clearly described, although without understanding their origin, significance and function.

There is general agreement that Giulio Bizzozzero was the first, in the years 1881 -1882, to establish their central role not only in physiological haemostasis, but also in thrombosis. It is noteworthy that the discovery of blood platelets and their function took place when the knowledge about the mechanisms of blood clotting was still largely incomplete. In fact, of the molecules involved in this process, only thrombin (1) and fibrinogen (2) had already been identified, although not characterized. Prothrombin was discovered by Pekelharing (3) only in 1892, the role of calcium by Arthus in 1890 (4), and the classical scheme of coagulation was definitively described by Morawitz in 1905 (5).

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In this article a brief history of the discovery of blood platelets is presented, with a particular emphasis on the fact that, in this case as well as in many other episodes in the history of science, many saw but only one understood.

The «small blood corpuscles» before Bizzozzero

After the well known communication presented by Leewenhoeck at the Royal Society of London (6), Hewson (7) was probably the first to fully describe in 1780 very small undefined particles in blood. Alfred Donne (1801 - 1878) (8), a French histologist, named in 1842 «globulin du chyle» (that is to say small globules derived from plasma) a sort of small globular, pale, opaline corpuscles visible in blood. The same corpuscles were later described by Beale in 1850 as particles of «germinal matter» *(Bioplasma kornchen)* and by Zimmermann in 1860 as «small corpuscles» *(Korperchen)* (9).

No better description was provided by these authors. The first careful description of those blood components later named *«piasthne»* by Bizzozzero was made by Max Schultze (1825-1874) (10) who, in 1865, wrote: «...clumps of irregular shape and different size, up to 80 u., composed by small globules or colourless granules, having a diameter of 1-2 \i....These particles are often sharp-cornered or have a granular appearance....lack spontaneous motility... radial extensions may be seen starting from the periphery of these clumps....related to blood clotting.... as filaments of coagulated fibrin». To this merely morphological observation no experiment followed to clarify the nature of the observed vesicles. However, as they showed a granular appearance, Schultze, with the over-simplified attitude typical of his time, inferred they had to be considered degenerate and disintegrated leukocytes.

As Schultze was in high favour with the contemporary haematologists, many investigators, such as Riess in 1872 and Laptschinski in 1874 (11), confirmed leucocytes, mainly during infectious diseases, as the origin of the Schultze's corpuscles. However, in the same years other authors considered them as clots of fibrin (Ranvier in 1873) (12) or as a particular kind of bacteria (Osier and Schafer in the same year) (13).

Acleardescription of the Schultze's «vesicles» was published by George Hayem (14) between the years 1878 and 1879. He wrote: «Among red and white cells small corpuscles are visible, having at the beginning the appearance of very delicate and pale erythrocytes. They rapidly modify their shape, becoming spinous and adhering to the glass...showing a tendency to stick to other similar corpuscles and to form aggregates.... their diameteris between 1.5and4.5|i...theiryellowish colour can become more intense and reddish by absorption of haemoglobin»; Hayem was a renowned haematologist of his time and was an expert on haemolytic anaemias, in which the presence of polymorphous, spiny or fragmented red cells can be often observed. This was probably the reason, together with the observation that these elements were often coloured by haemoglobin, for his erroneous conclusion that these were related to erythrocytes and to be considered as their precursors, justifying the term «haematoblasts». The Hayem's hypothesis was rejected by Riess, who confirmed in 1879 the origin of the particles from leucocytes, and by Neumann who, in 1880, stated they were artifacts derived from red cells following an incorrect technique of venipuncture (15).

The work of Bizzozzero and the discovery of platelet function.

The Schultze's and Hayem's corpuscles are the third morphological element of blood, independent of white and red cells.

Giulio Bizzozzero (1846-1901) was thefirst, in 1882 (16), to clearly establish the significance of the particles whose nature had excited so many debates. By means of *in* v/Voand *in vitro* investigations, he demonstrated they were visible not only in blood extracted from veins, but also in circulating blood observed by use of an Hartnack microscope in the mesentery of living animals, both in veins and in arteries (so excluding that they were artifacts depending on the arrest of circulation). Hefirmly stated thatthese corpuscles were the third morphological element of blood, totaly unrelated to white and red cells. Of these cells Bizzozzero gave a more precise description than other authors, reporting an oval or round, almost always lenticular shape (and not biconcave as stated by Hayem, leading him to erroneously identify them as precursors of red cells). The diameter was also more precisely reported, as between 2 and 3 u.. Both in the vessels after the circulation was stopped, and *in* w'frowhen blood extracted from the vein was observed, these elements underwent rapid morphological change, emitting long protrusions within 2-3 minutes and forming granular aggregates as large as 80-100 |i with other elements, similar to observations by Schultze and Hayem. Bizzozzero confirmed their

adhesive properties, not only to glass but also to the damaged vasculature. Moreover, he clearly stated they did not contain haemoglobin nor nuclear material, and therefore they could not be considered as precursors of erythrocytes. Bizzozzero named these elements *«piastrine»,* i.e. small plates (later platelets) and in 1882 proposed a translation into German as *«blutplattchen»* and into French as *«petitesplaques»* (later *plaquettes)* (17). The staining of platelets with a solution containing sodium chloride and methyl violet showed that they were formed of two components, one pale, hyaline, scarcely coloured (later named hyalomere) and another granular, coloured, bright (later named granulomere).

However, the greatest merit of Bizzozzero was the understanding of the platelet role in haemostasis and thrombosis. The mechanism of formation and structure of white thrombus had been extensively studied by several investigators. Long before Bizzozzero, in 1856 Virchow had described the initial thrombus as constituted only of clotted fibrin and leukocytes. This opinion had the agreement of Paolo Mantegazza, professor of General Pathology at the Pavia University and the first mentor of Bizzozzero, who in 1869 had performed a famous experiment demonstrating that a thread introduced in a vein of a living animal, once extracted, appeared to be coated by a whitish material composed of fibrin and leukocytes. In 1871 Rahn had also confirmed that leukocytes were the main component of white thrombi. Moreover, Weiger in 1877 and Cohnheim (who was unacquainted with the Bizzozzero's work) in 1882 had stated that the formation of fibrin clot itself was due to the action of leukocytes (19).

Bizzozzero dealt with this problem by investigating in vivo, with a microscope, what happened in small veins or arteries whose internal surface had been damaged by a needle. After a short time platelets adhered to the vessel wall, changed their shape by emitting protrusions of various lenght, then induced the aggregation of other elements, including a few red and white cells, until the formation of a network of fibrin fibrils in which platelets lost the appearance of distinct cellular elements. Here are some conclusive sentences derived from his monograph published in 1883 (20): «The thrombus material is constituted by few leukocytes plunged in large masses of platelets»; «This thrombotic material may be of a great value in stopping haemorrhages by closing discontinuities in a vessel wall» (this is the first clear statement in the history of medicine of the physiological role of platelets in haemostasis); «The granular disintegration of platelets is the unique change that one can observe in the morphological elements of blood affected by the preserving action of the vessel wall»; «Fibrin precipitates only where platelets are accumulated"; and finally «The main role in blood clotting is to be ascribed to platelets and not to white cells».

After Bizzozzero

German and French papers were published in 1882 and a lively and sometimes violent discussion followed. Some authors, such as Norris and Neale (21), claimed an undemonstrable priority about the observations presented by Bizzozzero, while others, such as Schmidt (22) persisted in maintaining the idea that platelets derived from disgregated leukocytes, and that leukocytes and not platelets were the thrombusforming elements. Moreover, Bizzozzero had a severe dispute with Hayem, who communicated in Paris in 1882 some of Bizzozzero's results as his own observations. It was only ten years later that absolute priority in discovery of platelet function in haemostasis and thrombosis was generally given to Bizzozzero.

Among so many merits, one failure may be ascribed to Bizzozzero. He had extensively investigated the bone marrow histology (23), and had the intuition that platelets also, as erythrocytes and leukocytes, could originate from this tissue, but he was unable to prove his

hypothesis. A justification may be found if one considers that Bizzozzero, in the last ten years of his life, (he died of pneumonia in 1901), became an authoritative personage, president of medical, microbiological and hygienical societies and, from 1895, Senator of the Kingdom of Italy, that allowed him frequent visits to Rome and other political engagements. It was only between 1906 and 1910 that bone marrow megakaryocytes were identified as precursors of blood platelets by Wright (24).

Afterthe work of Bizzozzero and the discovery of megakaryocytes by Wright, few advances in the knowledge of platelet biochemistry and pathophysiology occurred until the year 1960, when platelet agonists started to be characterized. Hellem and Owren discovered, the aggregating role of ADP (25), Hovig in 1963 that of collagen (26), and in 1966, Ardlie that of epinephrine (27) and Kloeze that of prostaglandins (28). The invention of the aggregometer by Born in 1962 (29) provided a valuable instrument to study platelet function and responsiveness to agonists *in vitro* and *ex vivo,* while two years later David-Ferreira published the first paper concerning platelet ultrastructure analyzed by means of electron microscopy.

The last thirty years have seen the definition and characterization of many platelet receptors, the analysis of molecular mechanisms involved in signal transduction and, above all, the introduction in medicine of anti-platelet treatments aimed at the primary and secondary prevention of atherothrombotic disorders.

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