Uniśkiewicz, Bogusław

Sources of Controversy in Blood Sedimentation Tests. Perspectives of the Edmund Biernacki's Method

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Bogusław Uniśkiewicz (Poland)

SOURCES OF CONTROVERSY IN BLOOD SEDIMENTATION TESTS. PERSPECTIVES OF THE EDMUND BIERNACKI'S METHOD

I

The term of blood sedimentation refers to the phenomenon of gravitational settling of the red blood cells (lat. *sedimentum* = sediment) with the release of liquid plasma, which collects in the upper part of the column of liquid. The estimation of the sedimentation speed is attributed to be of high importance for the diagnose and prognosis of the course of disease, and is therefore one of the most commonly performed routine tests.

Generally Robin Fåhraeus (1888–1968) is considered to have invented the method of estimating the sedimentation speed. However, the first one to have achieved that was in 1897 Edmund Biernacki. It is worth emphasizing, that Fåhraeus himself clearly pointed out[1] the important differences between his method of *stability of the blood suspension*, that is marking the linear sinking speed of the red blood cells, and Biernacki's *blood sedimentation speed,* which is the rate of voluminal separation of blood components. In the nineteen–twenties the distinctions introduced by the Swedish scholar were disregarded, and a sign of equality was placed between those two depictions. The chaos of concepts arisen as a result of that lasts until today. But the thing is not so much in the matter of precedence, which may be considered as only an issue of prestige, devoid of importance for science and medical practices, but rather – and probably: first of all – in the essence of sedimentation. In the present article I want to perform the historical recapitulation, and later on a content–related summary, placing verifiable facts before interpretations and opinions.

II

Some authors assume that sedimentation tests were run already in antiquity. It is not exact, because ancient investigators could not have known about the sinking of red blood cells, then impossible to observe. They did however notice that unvesseled blood does not always form a uniform clot. The spontaneous separation into layers occurring in some illnesses they treated as discrasia – separation of defectively mixed liquids (humours), which in

[1] Cf. R. Fåhraeus, *The suspension stability of the blood* in: *Acta Medica Scandinavica* 55, 1–2/1921, pp. 1–228.

accordance with their appearance were given names of: *black bile, blood, bile* and, the most interesting one, a layer of inflammatory coating on the surface – *phlegma* (flame). The notes on those observations come from Hippocrates (5th – 4th century). From the 2nd century after Christ the *crusta phlogistica* (inflammatory coat) functioned for *phlegma,* taken from the treatises of Galen, the doctor of Roman emperors. Galen suspected it to be the cause of inflammatory illnesses (phlegmasia) and propagated aggressive cleaning treatment by the use of bloodletting[1].

In 1674 the phenomenon of gravitational sinking of the red blood cells was described by Antonie van Leeuwenhoek. He characterized accurately the conditions for their circulation in vessels, and even drew conclusions on the possible pathological mechanisms. Although he did not create the terminology, he did characterize precisely the phenomenon of sedimentation. He stated that the sinking of blood cells occurs *in vitro* thanks to their flexibility, which *in vivo* allows getting through the capillaries, which conditions keeping up the vital actions of circulation. In years 1674–1678 he performed several observations of his own blood, and described them in 4 letters[2] to the Royal Society. He wrote among others: *Red pellets of blood* (...) *are heavier than the crystalline liquid including them* (...) *after releasing from the veins they settle slowly towards the vessel's bottom* (...) *and because of their softness and flexibility they lie down on one another* (...) *those pellets must be very flexible and soft in a healthy body, because they must flow through* (...) *capillaries and must therefore accept an oval shape, later on they return to the shape of a ball, passing through to broader spaces.* (...) *I wonder if some illnesses and maybe even death itself were not caused by the stiffness of those pellets.* (...) *To the agglomeration of those pellets I ascribe preceding clots.*[3] Leeuwenhoek thus presented the first in history compact display of blood rheology[4] (hemorheology) regarding mostly the microcirculation[5].

Curious is Leeuwenhoek's conviction about the high importance of the passage of blood cells through capillaries. Yet he didn't know their main physiological function as carriers of oxygen, which element was discovered only 100 years later (Scheele 1771, Priestley 1774). Although there were passed around over Europe the translations of the works of Michał Sędziwoj, died in 1636 (53 editions until the end of the 18th century), in which he was writing about a gas escaping by frying saltpeter and being an element of life. He even

[1] The conviction about instantaneous after–meal regeneration of blood could lead more eager doctors to exceeding the limits of safety. Only the 19th century overcame this problem. The uselessness of blood letting in pneumonia was proved in the half of the 19th century by Dietl – president of Cracow and propagator of sub–Carpathian health resorts, mentioned by Fåhraeus as a German.

[2] Cf. A. van Leeuwenhoek, *Recueil d'experiences et observations sur le combat qui procède du mélange de corps* [*Observations faites avec le microscope sur le sang et sur le lait, et communiquées à Mr. Oldembourg, sécretaire de la Société Royale de Londres, par Mr. Leeuwenhoek de Delft en Hollande*], Barthélemy Girin, Paris 1698.

[3] A. van Leeuwenhoek, *Recueil d'experiences et observations ...* .

[4] Rheology – science of flows and deformations.

[5] It is surprising that those so simple and basic for life correlations are not included in the canon of medical science, and are rather the margin of the pathophysiologists' research.

stated that it exists in the air as *a subtle saltpeter of philosophers*[1]. Sędziwoj's output was used by physicists formulating the gas rules. It is not sooner than in the 19th century, when the issue of transporting oxygen through blood was solved.

Over the following two centuries research upon the mechanics of blood did not take a step forward until the works of Biernacki, who was the first to perform the estimation of sedimentation rate. But the disturbances in the mechanics of erythrocytes did not raise interest among pathophysiologists until the second half of the 20th century.

III

Edmund Faustyn Biernacki (1866–1911) began his independent research already as a student of the University of Warsaw. His first scientific study (1887) he dedicated to the issues of fermentation. In years 1890–1891 on a scientific expedition he acquainted himself closer with the research and clinical output of Charcot, Hayem, Erb, Riegel and Kühne[2]. He was interested in the problems of metabolism in the widest extent – from the digestive processes to the gas exchange. His youthful optimism and successes in the field of science allowed him, for some time, a financial light–heartedness. Financial competition prizes he achieved one after another.

The huge Koczorowski prize of 3 thousand silver rubles (equal to ten yearly scholarships) Biernacki won with his work on blood gases[3], published in 1895. It required an enormous energetic effort (200 pages of experiment descriptions, compilations and personal hypotheses, 266 entries of world–famous works). In regard to the issue of oxygen transport he looked for a way of estimating the relative capacity of red blood cells in full blood. In Hedin's method of centrifugal hematocrit[4] he suspected the risk of damaging the blood cells, and he obtained his own way[5] of their gravitational settling by the use of various anticoagulants (agents for stopping coagulation of blood), from among which he considered powdered sodium oxalate to be the best.

The height of the measuring part of Biernacki's diagnostic cylinder amounted to 20 mm for 1 ml of blood (diameter about 8 mm). After various attempts he accepted those proportions as best for an efficient course of the

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[1] R. K. Meissner, J. M. Hasik, *Polski wkład w medycynę światową*, KAW, Poznań 1989

[2] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca metody opadania krwinek czyli tzw. odczynu Biernackiego* in: *Przegląd Lekarski* 19/1947, pp. 656–658 & 20/1947, pp. 696–700 & 23/1947, pp. 811–815 & 24/1947, pp. 852–856.

[3] Cf. E. Biernacki, *Przyczynki do pneumatologii krwi ludzkiej* in: *Pamiętnik Towarzystwa Lekarskiego Warszawskiego* 3/1895, pp. 535 sq., 4/1895, pp. 723 sq., 1/1896, pp. 11 sq.

[4] This term defined separating blood cells from the plasma by centrifuge. Nowadays the term *hematocrit* applies to the capacity of red blood cells expressed as the percent of full blood.

[5] Cf. E. Biernacki, *O stosunku osocza do ciałek czerwonych i o wartości różnych metod oznaczania ogólnej objętości krążków* in: *Pamiętnik Towarzystwa Lekarskiego Warszawskiego* 90, 1894, pp. 32–72, E. Biernacki, *Ueber die Beziehung des Plasmas zu den rothen Blutkoerperchen und ueber den Werth vershiedener Methoden der Blutkoerperchenvolumenbestimmung* in: *Zeitschrift fuer Physiologische Chemie* 19, 1894, pp. 179–224, E. Biernacki, *Blutkoerperchen und Plasma in ihren gegenseitigen Beziehungen* in: *Wiener Medizinische Wochenschrift* 36 & 37, 1894.

process and clear–cut evaluation of the result[1]. A tight stud enabled mixing the material with the anticoagulant by repetitive turning of the cylinder by 180° across the long axis. After placing the vessel in the upright position he read the volume of the released plasma after 30 min., after 60 min. and after the final settling of the blood cells (usually about 24 hours)[2].

Biernacki noticed a considerable variety of the sedimentation process' dynamics within the first hours of the test. Marking the volume of the upper layer of the released plasma in several moments as the percentage of its final volume, he achieved *the curve of sedimentation* as a characteristic of the process' course. It was a simple and clear–cut way of the results' relativisation to the hematocrit[3]. The way of testing ensured the calm settling of blood cells with no upward backflow of plasma and also no influence of eventual accidental deviation of a vessel from the perpendicular on the test result. Between the upper layer of the released plasma and sinking blood cells there appeared a sharp interphase, not evoking doubt as to the way of reading. Those qualities may induce attempts of including the method into the today's medical practice[4]. However, its postulated actualization requires decreasing the time–consumption. If the cylinder dimensions are standardized well, arduous calculation of volume may be replaced with the height of the column of liquid. Instead of calculating the plasma's volume percentage, a table of standards may be drawn for different hematocrits (e. g. every 5 % in the range between 10% and 90% (extreme pathologies)). 24–hours tests may also be replaced by simultaneous marking of the hematocrit by the use of a different method. A detailed development of those issues would require a separate report.

In Biernacki's tests usually around 25% of the plasma was released after 30 min. and 50 % after an hour – and those values he took for normal. The whole process until the *final sediment* lasted 24 hours, sometimes even more. So, as opposed to the present–day measurements of the linear sinking speed, for Biernacki the volume separation of components was important, expressed in the *sedimentation curve*[5]. Apart from the accelerated and slowed down sedimentations, Biernacki described also the *transitional* type with initial coagulation[6] present for some time, after which the dissolution of gel and a very quick sedimentation appeared.

Sedimentation research Biernacki published in three consecutive articles,

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[1] Cf. E. Biernacki, *Samoistna sedymentacya krwi jako naukowa i praktyczno–kliniczna metoda badania* in: *Gazeta Lekarska* 36/1897, pp. 962–968 & 37/1897, pp. 996–1004, E. Biernacki, *Die spontane Blutsedimentirung als eine wissenschaftliche und praktisch–klinische Untersuchungsmethode* in: *Deutsche Medizinische Wochenschrift* 48/1897, pp. 769–772 & 53/1897, pp. 847–849.

[2] Cf. E. Biernacki, *Samoistna sedymentacya krwi ...* , E. Biernacki, *Die spontane Blutsedimentirung ...* .

[3] Winrobe – Landsberg logarithmical curve for correction from year 1935, which was supposed to serve this purpose, was not accepted because of the excessive complications.

[4] Cf. B. Uniśkiewicz, *Odczyn Biernackiego i jego losy w ciągu stulecia* in: *Polski Tygodnik Lekarski* 47, 42–43/1992, pp. 980–982, B. Uniśkiewicz, *Zagadka OB* in: *Pamiętnik Towarzystwa Lekarskiego Warszawskiego* 6 (138), 2002, pp. 93–112.

[5] Currently the meaning of the sedimentation curve in methods of estimating the linear speed are being analysed, but as a purely theoretical issue – with no application into praxis.

[6] Clotting of blood because of the jellification of the plasma's proteins.

simultaneously printed in the Polish, German and Austrian press. The first one[1] included a preliminary mention of the method. The second[2] related to marking the relative volume of the *red sediment* in full blood. The third[3] presented the final technical variant of the method, as well as the sedimentation rate tests results in 70 cases of fixed etiology. All those works are mentioned in the main paper by Robin Fåhraeus[4].

Publications speak of the huge amount of work executed in that time by Biernacki. The sedimentation method, from an accidentally taken up idea to the executive standard, he presented in years 1893–1897[5]. In 1895 he published *Przyczynki do pneumatologii krwi ludzkiej*[6]. Various references in *Przyczynki ...*, as also the chronology of the mentioned publications point out the genesis of the sedimentation method as initially the auxiliary technique in gas analyses of blood (gasometry).

In years 1898–1899 Biernacki presented the sedimentation method at the sittings of the Warsaw Medical Society. He evoked criticism upon himself as well as on the whole of his scientific output[7]. Motives for that attack remain uncertain until today[8], but it was impetuous enough to induce him to changing his job and his country of dwelling[9].

In Germany Ernst Grawitz was interested in Biernacki's method; he related it in his works *Klinische Pathologie des Blutes* and *Methodik der Klinischen Blutuntersuchungen*. Besides, he commissioned O. Müller to do detailed research, which lead to his doctorate in 1898. Thanks to the contacts with the Hayem clinic in Paris, Biernacki could also present his method in Collège de France. In 1900 G. Marcano related it at the 13th Medical Congress[10], and in 1901 he presented it in an article on the methods of testing of the relative volume of erythrocytes in blood[11]. The nowadays commonly accepted Hedin's hematocrit obtained with the centrifuge method, based on the lactocrit used in dairy industry, was then not a routine method. The investigations and discussions lead then on the topic have become forgotten enough for some

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[1] Cf. E. Biernacki, *Badania nad składem chemicznym krwi w stanach chorobowych* in: *Gazeta Lekarska* 13, 32/1893, pp. 814–821 & 33/1893, pp. 849–858,

[2] Cf. E. Biernacki, *O stosunku osocza do ciałek czerwonych ...* .

[3] Cf. E. Biernacki, *Samoistna sedymentacya krwi ...* , E. Biernacki, *Die spontane Blutsedimentirung ...* .

[4] Cf. R. Fåhraeus, *The suspension stability of the blood*, p. 62.

[5] Cf. E. Biernacki, *Badania nad składem chemicznym krwi ...* , E. Biernacki, *O stosunku osocza do ciałek czerwonych ...* , E. Biernacki, *Samoistna sedymentacya krwi ...* .

[6] E. Biernacki, *Przyczynki do pneumatologii krwi ludzkiej.*

[7] Cf. E. Biernacki, *Pro domo mea*, Druk Fr. Karpińskiego, Warszawa 1902.

[8] He was attacked, among others, because of writing a methodological work, *Istota i granice wiedzy lekarskiej*, Biblioteka Dzieł Wyborowych, Warszawa 1898.

[9] Cf. B. Uniśkiewicz, *Zagadka OB.*

[10] Cf. J. Barbier, G. Piquet, *La sédimentaion sanguine en pratique médicale courante*, Masson et C[ie], Paris 1946.

[11] Cf. G. Marcano, *La sédimentation sanguine et l'hémostéréometrie en pratique médicale courante* in: *Journal de Physiologie et Pathologie Générale* 1901, pp. 167–182.

erudites on the subject to mention Marcano as the ... predecessor of the sedimentation method. Froment states[1], that Gilbert and Weill performed a modification on the method, and their work was corrected and completed by Claude in 1908. According to the same source *sedimentation was reborn from the ashes in 1913 and was this time not forgotten. It is interesting, that its repeated appearance took place in the veterinary applications thanks to a French vet Cesari.* In 1925 the method entered medicine in the modification by Cesari – Cordier – Chaix[2].

In 1904 Biernacki achieved his habilitation degree at the University of Lvov and became a private reader (with no salary) in the department of general pathology[3]. In this time he also managed to make a few translations (among others J. L. Sonderegger's *Podstawy ochrony zdrowia* [*Basic health protection*] and I. Miecznikow's *O naturze ludzkiej* [*The outline of blood pathology*] as well as prepare for print *Zarys patologii krwi*, published in Warsaw in 1906[4]. Despite difficult conditions – he initially makes a living by fulfilling the function of a *bath doctor* in Karlsbad, in holiday periods[5] – he returned in Lvov to the interrupted research threads. In the field of sedimentation he published a micromethod for testing frogs' blood (1906). In 1907 he published together with his assistant T. Hołobut a work[6] on the influence of transitory outside temperature changes on the sedimentation and morphology of blood in rabbits and frogs. It may be added that Hołobut occupied himself also with the influence of blood pressure on its morphology, what now reappears as a topic of hemoreological works.

In 1908 Biernacki obtained the title of associate professor, and thus better working conditions, although his health had already deteriorated. He died suddenly on December, 29, 1911. He left 97 scientific works[7], whose input into the Polish and world medicine has never become accurately and adequately evaluated. Almost the whole of his output fell into oblivion. In the world medicine only *objaw Biernackiego* (Biernacki's symptom)[8] remained – the anesthesia of the ulnar nerve in the course of the spinal cord tabes. The sedimentation method got caught on in a deformed shape, without preserving the name of the author. In Poland after his name[9] it was called *odczyn Biernackiego* (Biernacki reaction, in short OB). The sinking test is commonly considered a routine, applied to all in–patients. But no one has ever taken up the

[1] Cf. J. Barbier, G. Piquet, *La sédimentaion sanguine* ... .

[2] Cf. J. Barbier, G. Piquet, *La sédimentaion sanguine* ... .

[3] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca* ... .

[4] Cf. E. Biernacki, *Zarys patologii krwi*, Druk. E. Kowalewskiego, Warszawa 1906.

[5] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca* ... .

[6] Cf. E. Biemacki, T. Hołobut, *Blutveraenderungen bei thermischen Einfluessen* in: *Zeitschrift fuer experimentaele Pathologie und Therapie* 4, 1907, pp. 163–184.

[7] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca* ... .

[8] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca* ... .

[9] Cf. E. Biernacki, *Samoistna sedymentacya krwi* ... , E. Biernacki, *Die spontane Blutsedimentirung* ... .

methodological analysis of the application range of the primal executive variant, although the purposefulness of this undertaking is a logical consequence of the mentioned below ICSH postulates.

The same 1911 brought also Grawitz's death, as well as the probably last edition of his work on blood tests, presenting among others *Sedimentierung nach Biernacki.* From that moment on the sedimentation method began a life of *a phantom cruising around Europe,* which in year 1926 crossed the Atlantic Ocean thanks to Westergren's publication[1]. Barbier and Piquet[2] mentioned Cesari's method used in veterinary science in 1913, which after various modifications would enter medicine in 1925 as the Cesari – Cordier – Chaix method. In 1917 Ludwik Hirszfeld, remaining in Serbia because of the epidemic of typhus fever, proposed a method of sinking blood testing to diagnose malaria[3]. Using probably an accidental set of pipettes, he did not determine the exact vessel sizes, noting only that the sample's capacity amounts to 1 cm^3, same as by Biernacki.

IV

On years 1917–1918 fall the first works published in the Swedish magazine *Hygeia*[4] by a Swedish pathologist, Robin Fåhraeus (1888–1968), working in Uppsala, on the increased *agglutination*[5] of blood cells during pregnancy and several conditions of illnesses[6]. In 1921 he published[7] his own method of evaluating the erythrocytes' sinking speed, which he called the *blood suspension stability.* He performed his observations whilst working on the issue of *crusta phlogistica* of the ancient, already then known as *buffy coat.* He logically assumed that for the *crusta* to come into being, quick enough sinking of blood cells is necessary, measured in mm/h. *Crusta* did not last as an object of interest in the face of spreading morphological, biochemical and serological tests. However the mechanism of increased sinking remained a vivid topic for the investigations throughout long decades. In this area Fåhraeus managed to estimate the meaning of the blood cells' rouleaux formation[8], related in a closely undefined way to the amount of fibrinogen and other globulins. Often noticing the creation and disaggregation of rouleaux he deduced that to a certain limit this phenomenon is correct, and even profitable

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[1] Cf. A. Westergren, *The technique of the red cell sedimentation reaction* in: *American Revue of Tuberculosis* 14, 1/1926, pp. 94 sq.

[2] Cf. J. Barbier, G. Piquet, *La sédimentaion sanguine ...* .

[3] Cf. L. Hirschfeld, *Ueber ein neues Blutsymptom bei Malariakrankheit* in: *Correspondenz–Blatt fuer Schweizerische Aerzte* 47, 31/1917, pp. 1007–1012.

[4] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca ...* .

[5] Nowadays this term relates to the gluing together of blood cells in shapeless and firm lumps. Agglutination proves the existence of specific and strong gluing factors.

[6] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca ...* .

[7] Cf. R. Fåhraeus, *The suspension stability of the blood.*

[8] In static conditions red blood cells of preserved flexibility join spontaneously into long rows, bringing to mind coins in rolls. As opposed to agglutination, they are impermanent and sever easily under the influence of mechanical factors, e. g. the flow of the environment.

for the hemodynamics: *Each healthy blood forms rouleaux and sedimentates with a certain speed*[1]. *Crusta vel buffy coat* formed on the sample's surface the easier, the faster was the sinking of the blood cells. Thus the increased linear sinking speed would express the intensity of the inflammatory condition. Further years of his life Fåhraeus dedicated to hemoreologic research, as well as organizing works on creating, and later on developing the actions of the International Hemorheological Society, which was formed in 1966 at the founding convention in Reykiavik[2].

As it was mentioned earlier, Fåhraeus knew Biernacki's works, that he cited[3]. Methodological scrupulosity ordered him to differentiate clearly his own method of *the blood suspension stability* and Biernacki's sedimentation, the precedence and distinctness of which he indicated clearly in his work: *It would be well to differentiate here between what the older scientists and I myself have meant by the sinking speed of the cells and what Biernacki meant by the speed of sedimentation, the term which this writer most often employs and which in this account I have reserved for him.*[4] This differentiation was ignored. Fåhraeus was called the *rediscoverer of sedimentation*[5] and this way of quoting remains until today in the world leading literature, although it is absurd in the light of his own statement quoted above.

The causal mechanism of the accelerated sinking was by Fåhraeus' followers ascribed solely to plasma factors, with a total omission of the possibility of the existence of cell–factors. Fåhraeus' followers ignored also the final fragments of the mentioned work[6] presenting the results of tests upon material subjected to thermal processing. Those tests showed clearly the influence of cell–factors on the sedimentation process. However Fåhraeus himself abandoned this research thread, moving over to the observation of blood cells in the flow.

Described by Fåhraeus changes of consistence, form and colour of the *buffy coat* as a subject for observation during the course of inflammation were not adopted in the face of the then already strong position of biochemical and immunological research. The evaluation of the linear sinking speed did however gain numerous followers, although the original methodology could not enter medical practice because of a considerable volume of required blood sample. The early 20–ies brought the origin of many modifications, concerning the limitation of the sample's capacity by using pipettes of a very small inner diameter.

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[1] R. Fåhraeus, *The influence of the rouleaux formation of the erythrocytes on the rheology of the blood* in: *Acta Medica Scandinavica* 161, 1958, pp. 151–165.

[2] Cf. A. L. Copley, *The Robin Fåhraeus memorial lecture at Seventh International Congress of Biorheology, Nancy, France, 18–23 June, 1989* in: *Biorheology* 26, 3/1989, pp. 423–461.

[3] Cf. R. Fåhraeus, *The suspension stability of the blood*, p. 62.

[4] Cf. R. Fåhraeus, *The suspension stability of the blood*, p. 64.

[5] He was probably supposed to be the *reinventor* of a forgotten phenomenon, which was first interpreted by Hippocrates.

[6] Cf. R. Fåhraeus, *The suspension stability of the blood.*

A sharp rivalry began among the modificators over precedence and domination (Linzenmeier, Plaut, Panczenkow, Westergren[1], French scientists). Ultimately most common became Westergren's method. With the time flow various reservations of technical nature appeared. French scientists (Badin[2] among others) noticed the blurring of interphases, occurring in narrow and long pipettes. Responsible for this was the plasma upward backflow, evoked by blood cells sinking in the high column of liquid. Following experiments[3] proved that the backflow upsets the harmonious agglomeration of blood cells, tearing apart the previously created rouleaux and carrying sometimes the loose blood cells to the surface of the column of liquid. The backflow effect was changing considerably already during the tube's tilt by a couple of degrees from the perpendicular, which is not always possible to control. Nonetheless, this way of evaluating the sinking speed was diffused under the term of *blood sedimentation* (*Erythrocyte Sedimentation Rate* – ESR or *Blood Sedimentation Rate* – BSR). In 1943 Fuente–Hita[4] applied the pipette's inclination under the degree of 45° canalizing the backflow beneath the upper wall, which accelerated 4 times the sinking of blood cells sliding down on the lower wall. There appeared doubts as to *the wall's effects* (the blood cells' adhesiveness, chemical influence, etc.). This way of testing is seen nowadays in the modification by Pronto.

The meaning of slow sedimentation began drawing attention of a growing number of scientists. In the 70–ies Rampling and Sirs proved[5], that it involves a diminished flexibility of erythrocytes, and so it may express their worse flow through capillaries. In 1983 Chien and Jan[6], using electron microscopy of blood cells forming rouleaux in artificial dispersal environments affirmed that the cause of rouleaux formation lies in building cell–to–cell bridges by adhesion of stringy macromolecules to the erythrocytes' surface, where the efficiency of rouleaux formation increases along with the growing concentration of those molecules and the increase of the erythrocytes' flexibility. In the same year Syoten Oka[7] expressed the sedimentation speed in the form of a three–variable formula: the hematocrit, the plasma's viscosity (representing the concentration of the agglomeration factors), the erythrocytes' flexibility. In following plans he assumed examining the causes of the clinical data's departure from this rule, yet he did not manage to accomplish his research before his death.

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[1] Cf. A. Westergren, *Studies of the suspension stability of the blood in pulmonary tuberculosis* in: *Acta Medica Scandinavica* 54, 3/1920, pp. 247 sq.

[2] Cf. J. Barbier, G. Piquet, *La sédimentaion sanguine ...* .

[3] Cf. P. Snabre, P. Mills, *Structural changes of sedimentating blood suspension* in: *Biorheology* 20, 4/1983, pp. 427.

[4] Cf. J. Barbier, G. Piquet, *La sédimentaion sanguine ...* .

[5] Cf. M. W. Rampling, J. A. Sirs, *Fibrinogen, fibrinogen degradation products and erythrocyte flexibility* in: *Thrombosis and Haemostasis* 38, 2/1977, pp. 668 sq.

[6] Cf. S. Chien, K.–M. Jan, *Ultrastructural basis of the mechanism of rouleaux formation* in: *Microvascular Research* 5, 2/1983, pp. 155 sq.

[7] Cf. S. Oka, *A physical theory of erythrocyte sedimentation* in: *Biorheology* 20, 4/1983, pp. 397 sq.

V

In Poland the Biernacki's method was forgotten (although the term *OB* (in Polish *odczyn Biernackiego*) – Biernacki's reaction – is still in use and refers to all methods of observation of red cells sinking). Under the term of *sedimentation* there appeared from abroad methods of estimating the linear sinking speed. The issue of blood sedimentation finally became considered only in 1923 at the 5th Polish Internists' Convention in Vilnius[1]. Eleonora Reicher resigning from the presentation of her own work (based on the Plaut's method) proposed commemorating Edmund Biernacki as the discoverer of sedimentation[2]. Gluziński suggested an adequate content–related term *Biernacki's method.* It was the most proper proposal as it comes to the relation to contents. Finally the unfortunate term of *Biernacki reaction* was accepted, one leaving too much freedom of interpretation. It encompasses in its range all possible sinking tests and allows mentioning the name of the author of the executive variant next to the name of the inventor, what covers up the methodical contradictions. For example the author of the present article was taught of *Biernacki reaction by Westegren method.* In this way, although Biernacki's name was commemorated, the issues of the distinctiveness of his method, its cognitive virtues and the range of application were put aside. It is a common opinion in Poland that each and every method of sinking tests is an improvement of *Biernacki reaction,* this term functioning in Poland, whereas in other countries the term ESR is in use – or its equivalent in other languages.

After World War Two, in 1947 Adam Smoluchowski[3] proved Biernacki's precedence on the ground of abundant historical material. His work however did not evoke a lively response. Ten years later doctor Adam Rytel working in the USA presented in an English–language magazine[4] the outline of Biernacki's method. He proposed world–wide spreading of the term *Biernacki reaction*, but he did not get any response. In 1987, nearly 100 years after Biernacki's discovery, E. Kucharz put an occasional mention of Biernacki in the *Lancet* magazine[5]. Characteristic was the reader's voice in the discussion, pointing at the precedence of the antique Hippocratic writers, for whom 20 years of advance stops meaning anything.

VI

Summing up: Edmund Faustyn Biernacki is the discoverer of the volumetrical way of evaluating the dynamics of the formation of red blood cells

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[1] Cf. *Protokół III posiedzenia V Zjazdu Internistów Polskich 10 VII 1923* in: *Polskie Archiwum Medycyny Wewnętrznej* 4, 2/1924, pp. 42–43.

[2] It is hard not to notice that the historical penetration of Eleonora Reicher, later prof. of rheumatology, the founder of the Institut of Rheumatology in 1950, became the proverbial Ariadne's thread – the only way leading to the forgotten output of Biernacki.

[3] Cf. A. Smoluchowski, *Edmund Biernacki jako odkrywca ...* .

[4] Cf. A. Rytel, *The sixtieth anniversary of the development of the sedimentation test by Edmund Biernacki* in: *Polish Medical History and Science Bulletin* 1, 2–3/1957, p. 3.

[5] Cf. E. Kucharz, *Edmund Biernacki and the erythrocyte sedimentation rate* in: *Lancet* 1/1987, p. 696 (8534).

sediment, that is, following his own term, the *blood sedimentation rate* (1893–1897). Robin Fåhraeus on the other hand is the author of the *blood suspension stability* conception (1921). That those two approaches vary in an important issue was highlighted by Fåhraeus in 1921, who reserved the term *sedimentation* for Biernacki's method[1]. His postulate was however ignored. In the 1920–ies to the multiplying *linear* methods was ascribed the term of sedimentation. This contributed to a conception chaos and upsetting the values of the diagnostic method, and such state of vague interpretations remains until today[2].

Taking as the starting point Fåhraeus's differentiation between his method of blood suspension stability and Biernacki's sedimentation, one may draw a logical conclusion, that the latter is the only sedimentation method *in the strict sense,* but this argument is merely a formal subterfuge. Much more serious is the possibility of capturing the impeded (slowed down) sedimentation, expressing the deprivation of the blood cells' flexibility[3]. In this field Biernacki's method could prove useful in rheological tests. Its simplicity and low cost could enable the expansion of rheology from the field of theoretical investigations to the field of a broad clinical application. Such an expansion of the sedimentation's usage would put aside its history. Moreover, it would create a pragmatic way of avoiding the imposing deontological estimation of reprehensible actions of the scientific misconduct type, consisting in false quoting of Fåhraeus and omitting outputs of the publications ahead of his time. The emphasis set on future rheological usage of the method in Evidence Based Medicine that I am proposing assumes its exploitation as a simple way of estimating of some aspects of microcirculation. Some space should thus be reserved for this issue.

Until today the mechanics of microcirculation is usually reduced to the *play of capillaries* consisting in the changes in the arterioles' diameters, what could result from the primal character of structural–anatomical ideas before the action–physiological intuitions. Currently many scientists incline towards the former idea (*vide* Leeuwenhoek), saying that the erythrocytes' flexibility determines the flow through true capillaries[4]. Those two conceptions are not contradictory, rather complementary. If in the contractile arterioles of the diameter of a dozen or so micrometers the condition of vessels and the heart's pumping force determine the flow, then in the stiff capillaries narrower than

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[1] R. Fåhraeus, *The suspension stability of the blood*, p. 64.

[2] In Poland functions the term *Biernacki reaction*, but his method is forgotten, and in other countries *the erythrocyte sedimentation rate* is meant, but for methods deriving from Fåhraeus' *blood suspension stability.*

[3] Nowadays some researchers take up sedimentation slower than 5mm/h as *hampered sedimentation.* It is vague however, because it may be caused by plasma backflow evoked by vigorously falling erythrocytes, which gives the picture of *apparently slow sedimentation.*

[4] Cf. L. Dintenfass, *Theoretical aspects and clinical applications of the blood viscosity equation containing a term of the internal viscosity of the red cell* in: *Blood Cells* 3, 2/1977, pp. 367–374, T. Nakamura et al., *Rheologic and pathophysiological significance of red cell passage through narrow pores* in: *Blood Cells* 20, 1/1994, pp. 151–165, R. J. Weed, *The importance of erythrocyte deformability* in: *American Journal of Medicine* 49, 1970, pp. 147–150.

the erythrocyte's diameter (7–8 μm) grows the meaning of the blood cells' flexibility.

Basing on Fåhraeus' opinion about every healthy blood sedimentating at some speed[1], one should conclude that on the one hand the slowing down of the process could indicate the damage of blood cells, but on the other, that accepting *normals* close to zero shows only the unreliability of the used method. It is characteristic that not one of the current methods gives the norm's limit, below which the sinking is pathologically slow. This problem has been gotten rid of by accepting the unproved thesis saying that slow sedimentation does not have a diagnostic meaning, although this course of the process is ascribed to the conditions of cachexy as well as to the heart and lungs diseases[2], which was also observed by Biernacki[3]. An interesting view on the issue was presented by Ahlquist[4], who connected the sudden change in the test course during the history of illness from the very fast sinking to the extremely slow one with fibrinogen deficiency as a result of consumption due to intravascular coagulation, i. e. a state of emergency in the area of shock mechanisms, threatening the patient's life. An equally dangerous state in the inflammatory course is the impairment of the blood cells' flow through capillaries as a result of their stiffness, and this situation could be diagnosed by noticing a considerable slowing down of the sedimentation process (hampered sedimentation)[5]. The possibility of such diagnosis is allowed by Biernacki's method.

Findings coming from 80 years long investigations upon the sedimentation mechanism can be summed up in Biernacki's statement: *In those places where blood formed rouleaux, no poikilocytes*[6] *can be seen. And what may be the cause of poikilocytosis? It is probably the decreased flexibility of disks*[7]. It seems thereof that the stiff red blood cells which have lost their ability to regain spontaneously their disc shape, do not form rouleaux and individually fall slower than the flexible agglomerated cells, and in clinical categories give a worse flow through capillaries and may be inefficient in the gas exchange. Biernacki's intuition concerning the correlation between sedimentation and body–oxidation gains new sense in the light of the achievements of knowledge about the mechanical characteristics of blood as a liquid in flow (hemorheology). One should regret that the author of the observation mentioned above performed on a fresh drop of blood did not link it with the mechanism of sedimentation. It would prevent the method from distortion and misinterpretations.

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[1] Cf. R. Fåhraeus, *The influence of the rouleaux formation ...* .

[2] Cf. A. Westergren, *Studies of the suspension stability ...* .

[3] Cf. E. Biernacki, *Samoistna sedymentacya krwi ...* .

[4] Cf. J. S. Ahlquist, *Another pathologist's view on DIC and thrombosis on their relationship to the ESR* in: *Thrombosis and Haemostasis* 38, 2/1977, pp. 584 sq. [DIC = *disseminated intravascular coagulation*].

[5] According to Oka's formula (see above).

[6] From the Greek – differently shaped cells.

[7] E. Biernacki, *Zarys patologii krwi*, Druk. E. Kowalewskiego, Warszawa 1906, p. 59.

VII

What can be the future fate of sedimentation tests? The interpretation of the acceleration of sinking as the *inflammatory sensor* presented so far fails the chance to remain in the face of gaining more specific markers, among which the CRP protein is a huge success. Its concentration correlates well with other markers, and even with the dynamics of atherosclerosis having its source, among others, in the inflammatory processes. Will then sedimentation become relinquished? Surely so, unless scientists return to the fundamental one[1], that is to the one described by Biernacki[2].

In 1988[3] the International Committee for Standardization in Haematology (ICSH) affirmed the impossibility of comparing results obtained from various sinking tests, and even from various material samples within limits of the same method (among others because of the difference of hematocrit, being one of the factors determining the phenomenon's dynamics). The status of the reference method of blood sedimentation accepted in 1977 was repealed[4]. In 1988 a postulate was put forward to draw up a new *reference method.* In 1993[5] this postulate was put forward again, this time along with pointing out the required technical details. It was suggested, among others, for the new method not to require diluting of blood and to comply with the dependence of obtained results on the variability of the hematocrit (relative capacity of red blood cells in full blood). A satisfactory solution has not yet been found.

Biernacki's method, which in 1997 was supposed to celebrate its 100th anniversary, meets both criteria. Why then it was not accepted by the ICSH? It may be interpreted in several ways, for example by its oblivion. Does it mean that the introduction of Biernacki's method in over 100 years after the publication of his final author version[6] should be demanded – clearly and in public? The facts presented above provide foundation for a categorical affirmative answer to this question. Serious indications to such option could be concisely formed as follows. Firstly, the cognitive considerations – the method has never been subjected to a thorough analysis in the aspect of the interpretation of results, its clinical usefulness, etc. Secondly, deontological considerations speak for it: the usefulness of generally used methods for the estimation of the patients' health has been challenged[7], and Biernacki's method was groundlessly and absurdly thrown aside from the scientific and clinical circulation. Thirdly, the simplicity of Biernacki's method reduces

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[1] Cf. B. Uniśkiewicz, *Zagadka OB.*

[2] Cf. E. Biernacki, *Samoistna sedymentacya krwi ...* , E. Biernacki, *Die spontane Blutsedimentirung ...* .

[3] Cf. ICSH, *Guidelines on selection of laboratory tests for monitoring for the acute phase response. ICSH expert panel on blood rheology* in: *Journal of Clinical Pathology* 41, 1988, pp. 1203–1212.

[4] Cf. ICSH, *Recommendations for measurement of erythrocyte sedimentation rate of human blood* in: *American Journal of Clinical Pathology* 68, 1977, pp. 505–507.

[5] Cf. *ICSH recommendations for measurement of erythrocyte sedimentation rate. ICSH expert panel on blood rheology* in: *Journal of Clinical Pathology* 46, 1993, pp. 198–203.

[6] Cf. E. Biernacki, *Samoistna sedymentacya krwi ...* .

[7] Cf. ICSH, *Guidelines on selection of laboratory tests ...* , *ICSH recommendations for measurement of erythrocyte sedimentation rate ...* .

nearly to zero the financial difficulties. Fourthly, it may open a new field for research in the disciplines of blood rheology, because it enables the evaluation of *hampered sedimentation,* thanks to which it may prove to be useful as a method of examining the erythrocytes' flexibility, conditioning their efficient flow through capillaries. On the other hand, one should fear that further marginalization and passing over in silence of Biernacki's discovery will decide unfavorably on the fulfillment of the drastic alternative: it will either come to putting Biernacki's method in practice, or a chaos of conceptions and interpretations in the field of blood sedimentation will dominate the following decades, harming both the patients and disorientated doctors. The choice of option should be given to the natural cognitive tendencies, the care about perfecting diagnostics, as well as to the deontological considerations, setting forth the respect for material truth.

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