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"Round, red globules floating in a crystalline fluid" – Antoni van Leeuwenhoek's observations of red blood cells and hemocytes

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ABSTRACT

This article addresses a deficit in the cell biology and hematology literature, specifically regarding Antoni van Leeuwenhoek's central role in observing and describing red blood cells and hemocytes. While the existing literature on the history of hematology usually mentions Antoni van Leeuwenhoek, typically, it is an incomplete summary of his contributions. Leeuwenhoek is cited as one of the three individuals who first saw and described red blood cells through their microscope lenses. Jan Swammerdam and Marcello Malpighi also documented red blood cells in human blood before Leeuwenhoek. The literature fails to mention that Leeuwenhoek commented on red blood "globules," as well as arthropod hemocytes, at least thirty-five times in thirty-one letters spanning thirty-nine years of correspondence to The Royal Society and others. Some of his descriptive passages were extensive. His observations on blood circulation are a separate set of observations in his letters and are not covered here. Leeuwenhoek viewed various creatures to see if there were characteristics to their blood cells that he could share with the recipients of his letters. He also would view the cells in different chemical and physical environments to understand their properties. Leeuwenhoek's observations of blood corpuscles are discussed in chronological order. Comments included in footnotes by the Committee of Dutch Scientists, who edited the published seventeen volumes of Leeuwenhoek's *Alle de Brieven*, are also discussed when their comments are relevant.

1. Introduction

Antoni van Leeuwenhoek's letters are essential documents in the maturation of science and historical microscopy. He described structures and objects that are typically sequestered within specific scientific disciplines. Leeuwenhoek often returned to subjects and samples discussed in earlier letters. Sometimes, it is due to his innate curiosity, perhaps because of an unresolved question that persisted from previous letters. At other times, one or more of his correspondents and colleagues had asked him for more details. Among his many areas of interest, fluid circulation — in plants and animals — and particularly the structures found in the blood of creatures, including mammals, fish, birds, amphibians, and arthropods, saw their share of his attention.

Leeuwenhoek did his best to explain, sometimes in metaphor, how the red blood cells — his blood globules or those of warm-blooded creatures — appeared to him. He also dissected several arthropods and made the first known observations of hemocytes in their hemolymph. Leeuwenhoek saw objects beyond his understanding but noted their peculiarities and moved on to other matters. He discussed his observations so thoroughly that there is little doubt left to the reader about what Leeuwenhoek has observed. In other cases, room for interpretation remains. The editors of the letters come to the reader's aid with comments on what Leeuwenhoek has reported.

Between 1673 and 1712, Leeuwenhoek's (*Alle de Brieven Collected Letters* Volumes 1 through 17) included at least thirty-five descriptions of blood globules and hemocytes in insects and crustaceans, structures that he described as colorless and "only less transparent than the fluid in which they floated" (Leeuwenhoek, 1967, p. 111) (Leeuwenhoek, 1979, p. 149). A comprehensive list of his blood and hemolymph observations can be found in Supplemental Material.

2. Recognition of Leeuwenhoek's contribution to hematology

Aside from a 1931 article by Dr. John Stein in the journal that preceded *Science* (Stein, 1931), the masterly work of Clifford Dobell (Dobell, 1932), and a section of F.J. Cole's (1937) review article (Cole, 1937, pp. 20–9), Leeuwenhoek has received scant credit for the extent of his observations of red blood cells and little mention for his notes on

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hemocytes. This oversight was addressed partially in Chapter 8 of *Antoni van Leeuwenhoek: Master of the Miniscule,* which focuses primarily on Leeuwenhoek's circulatory observations but includes some commentary on the blood corpuscles (Robertson et al., 2016, pp. 146–148).

Nonetheless, recognition of Leeuwenhoek's inquiries into the nature of red blood globules was not proportional to the scope of his work. In 1974, Douglas Surgenor edited a two-volume work called *The Red Blood Cell* and provided only the following description of Leeuwenhoek's work: "The description of the red blood cells of a man was finally accomplished in 1674 by Anton van Leeuwenhoek," followed by a brief quote from his letter to Henry Oldenburg from 7 April 1674 (James M. Stengle, 1974, p. 5).

Although his comments were limited, Dr. Maxwell Wintrobe, one of the 20th century's preeminent hematologists, is probably best at citing Leeuwenhoek's contribution. Wintrobe's first work on hematology came in *Blood, Pure and Eloquent,* a collection of essays addressing various topics in the field. In his opening essay, Wintrobe writes:

> The Dutchman Jan Swammerdam (1637—1680) and the Italian Marcello Malpighi (1628—1694) are often credited with describing the red blood corpuscles before Leeuwenhoek, but Swammerdam only referred to the presence of "ruddy globules" in the blood and doubted that blood in its vessels contains such globules, while Malpighi's interest seems to have been only casual. It is Leeuwenhoek, therefore, who deserves the credit for having provided the first real description of the red blood cells (Wintrobe, 1980, pp. 7–9).

Wintrobe goes on to state that "…the tiny constituents of the blood, the so-called red corpuscles (the erythrocytes), which carry oxygen about the body, were first seen; and it was with similar rudimentary equipment that the cells concerned with the defenses of the body … the "white" or colorless corpuscles (the leukocytes) may have been observed, perhaps first by Malpighi and by Leeuwenhoek…" (Wintrobe, 1980, pp. 10–11).

Several other essayists in *Blood, Pure and Simple* remark on Leeuwenhoek's contribution, yet none of them expand on the extent of his observations or mention his work describing arthropod circulation of hemocytes.

In *Hematology, the Blossoming of a Science,* Wintrobe adds to his 1980 work:

> The first complete account of the red cells was made by Antonj van Leeuwenhoek (1632—1723) of Delft. The hobby of this City Hall custodian was the grinding of lenses, which he did with exceptional skill; moreover he was curious and industrious and lived and worked until the age of ninety-one… Unlike Malpighi he did not mistake the red corpuscles for fat globules (Wintrobe, 1985, pp. 10–11).

It is worth noting that Malpighi recognized his error later in his life and realized that what he had thought were fat globules were blood cells, although he did not go further than this correction (Leeuwenhoek, 1939a, pp. 408–9).

Leeuwenhoek ekes out a bit more recognition in an article summarizing the achievements of Wallace H. Coulter, an innovator in cell counting technologies. Dr. Robinson, the author of the article, cites Dr. Stein's (1931) Leeuwenhoek article:

> Interestingly, key to Van Leeuwenhoek's role as an instigator of discovery was his ability to design and manufacture technologies — in his case a microscope-based one that was transformational in his time, being easy to manufacture even though rather difficult to use (Robinson, 2013, P. 24).

While these accounts provide some context to Leeuwenhoek's work and credit him with the "first complete account of the red cells," it significantly understates the scope of his contribution while placing the Swammerdam and Malpighi observations in perspective.

3. Leeuwenhoek and blood studies

The first letter mentioning blood was written to Henry Oldenburg, first secretary of the Royal Society and editor of *Philosophical Transactions* until 1677. The letter was dated 15 August 1673 and described the imbibition, digestion, and excretion by a louse of human blood. In the letter, Leeuwenhoek described interspecies (human-to-louse) blood circulation as a source of nutrition rather than an oxygen carrier (Leeuwenhoek, 1939b, pp. 55–57). Leeuwenhoek eventually will describe red blood cells' relative size, color, and shape, as well as hemolymph and hemocytes in various arthropods. He burrowed into the details of the circulation of blood fluid and red blood "globules" in many mammals, fish, frogs, and birds, although his numerous observations of blood circulation go beyond the scope of this review.

"Globules" is a term often used by Leeuwenhoek to describe structures he views through his lenses. In this single letter, he used "globules" to describe structures in blood, milk, breasts, hair, fingernails, and "unnatural tumors." He probably observed fat droplets in milk, breasts, and tumors, while he may have observed structures formed by proteins in hair and fingernails.

In a letter dated 5 April 1674 to Constantijn Huygens, Leeuwenhoek addressed some observations he had made during a visit to Huygens' house in Zuilichem "some time ago." Leeuwenhoek states that he.

> had observed blood from my hand, which I found to consist of red globules, also floating about in a wheylike fluid. This is to inform you that I have since examined my blood once more, this time with greater accuracy. I now find that the fluid in which the globules float are more like a crystalline than like a wheyish fluid" (Leeuwenhoek, 1939b, p. 67).

In a footnote the *Letters'* editors clarify that by writing "crystalline," Leeuwenhoek implies clear, whereas whey is turbid.

In a letter dated 7 April 1674, just two days later, he followed up with Oldenburg and confirmed the fluid's crystalline nature, but this letter adds some subtle details. He allowed that he has "divers times endeavoured to see and to know, what parts the Blood consists of" — he has observed blood and thought about it more times than he has written about in his letters. He writes that the "small round globuls" are "driven thorough [sic] a Crystalline humidity or water," that is, they are no longer floating but are driven, are in motion. He also said that when he views his blood cells in "very small parcels," they yield "very colour (weijnich couluer)," which is an archaic way of saying "little color" (Leeuwenhoek, 1939b, p. 75). Between his letter to Huygens and his letter to Oldenburg, Leeuwenhoek revealed several "new" observations about the blood, at first allowing that the globules are red, then that in small numbers they have little color.

On 24 April, Leeuwenhoek wrote to Huygens again. He shared that "four or five globules" have "very little colour" and noted that the globules "can only pass in one thickness and singly through some subtle veins," perhaps being his first observation of red blood cells passing through capillaries or arterioles feeding the capillaries. When the cells pass through these tiny vessels, "one cannot expect the red globules to impress the eye with the sensation of a red colour," thus stating that it is even less surprising when the cells are seen one by one.

In a 1 June 1674 letter, Leeuwenhoek responded to a series of questions from Robert Boyle, the eminent natural philosopher and Royal Society member. In 1684, Boyle would publish *Memoirs for the Natural History of Human Blood* and plan a revision (Boyle, 2000, pp. 3–102). Leeuwenhoek commented on blood color changes, the relative heaviness of blood globules compared to the serum in which they are suspended, and color differences of blood cells within the diameter of a vein. Leeuwenhoek then made nine numbered observations, the first of which addressed Boyle's question directly. The second response is related to Leeuwenhoek's methods for blood sample observation, to his first description of the relative size of blood globules to a fine grain of sand, and included a further clarification that the globules are "white and colourless" as single cells. Leeuwenhoek stated that "Mr. BOILE" advised him to continue pursuing the observations he has provided to the Royal Society and in particular anything more regarding "…the red, florid colour which blood acquires as soon as it is drawn from the veins and exposed to the air, and also to the blood under the surface, as being distinct from the other blood in colour." The entirety of his first observation is included below and responds directly to Boyle's queries while expanding on related matters. The third note discussed the behavior of blood globules in a gently heated capillary. The rest of the numbered observations are related to globules in other materials, particularly in various kinds of bone.

> The small Red Globuls in the Blood, formerly spoken of are heavier than the Crystalline liquor in which they are carried, because soon after that the Blood is let out of the Veins, those Globuls by little and little subside towards the bottom; and being made up of soft fluid Corpuscles, and many lying upon one another, they do unite themselves close together, and by this close conjunction the Blood that is under the surface alters its colour, and becomes dark-red or blackish, as I have observed several times: of which I take the reason to be, (with submission to better Judgments) that the Air cannot move every way round about the Globuls, and hits as 'twere against a close darkish body. Touching the Florid red colour of the surface of the Blood exposed to the Air, that comes, in my opinion, from hence, that the uppermost Globuls are not press'd, and therefore retain their nature, and the Globuls subjacent to the uppermost lye close together, by reason of which close conjunction the Light cannot penetrate through them, but is reflected, and so gives a greater light to, and about, the uppermost Globuls, than they had before the union of the inferiour Globuls; and [consequently it is this] that makes [their red colour brighter and more florid] (Leeuwenhoek, 1939b, P. 93).

This passage received footnoted comments from the editorial committee working towards publishing *Alle de Brieven* Volume 1. The first comment is from Professor Doctor Gerard Heringa, a histologist working at the time (the late 1930s) in the medical school at the University of Amsterdam. He interprets Leeuwenhoek's comment regarding the blood corpuscles uniting "themselves close together" as referring to the formation of ***rouleaux*** or stacks of blood cells and that these formations of blood cells result in the venous or deoxygenated color of the blood deeper in the vein. Heringa also comments that if Leeuwenhoek is referring to ***rouleaux*** as the reason why the blood "… that is under the surface alters its colour, and becomes dark-red or blackish," the explanation is unnecessary, as the deeper blood cells might have been exposed to less oxygen and the hemoglobin, unknown to Leeuwenhoek, would be in its dark, deoxygenated form. It is not easy to assess whether Leeuwenhoek's description and Heringa's explanation are aligned with each other, as Leeuwenhoek does not provide us with an illustration of the phenomenon.

Another footnote expounds on the translation of a single word - ***lucht***. Dr. Heringa and Judica Mendels, a "qualified teacher of Dutch in a secondary school, Amsterdam" introduce an alternative translation (Leeuwenhoek, 1939b, p. 375). While the Royal Society interpreted the passage to mean that Leeuwenhoek believed that "air" was responsible for the bright red color of blood near the surface of a sample, Heringa and Mendels believe that this was due to mistranslation of the word "lucht," which meant "light" in Leeuwenhoek's dialect (Leeuwenhoek, 1939b, p. 95). If they are correct, Leeuwenhoek was merely commenting on the surface blood reflecting light differently than the deeper portion of the sample, rather than on whether the surface blood was different in the presence of "air." Again, it is difficult to arrive at a definitive interpretation of what Leeuwenhoek saw or meant by his description.

There is much to note in this letter, but perhaps the most stunning statement comes with Leeuwenhoek's estimate that the red blood globules are "25000 times smaller than a [fine] grain of sand" (Leeuwenhoek, 1939b, pp. 103–105). In a footnote, Heringa and Dr. Abraham Schierbeek comment that this would put the diameter of a red blood cell at approximately 8.5 μm, "which strikingly agrees with the now accepted computation of 7,2″ (Leeuwenhoek, 1939b, p. 103). Leeuwenhoek goes on to affirm previous observations that blood cells "… when they are single, and stick within to the sides of the Glass-pipes, will appear white and colourless," along with minor statements regarding how blood cells rise in a gently heated capillary tube (Leeuwenhoek, 1939a: 103–5), and that "…bloody Globuls did issue from between…" muscle filaments observed in cow flesh (Leeuwenhoek, 1939a: 109). Schierbeek, a lecturer in the history and methodology of biology at the University of Leiden, a prolific author with over 150 books, brochures, and articles to his credit, became an essential biographer for both Leeuwenhoek and Jan Swammerdam (Luyendijk-Elshout 197).

One month later, Leeuwenhoek wrote Oldenburg on 6 July 1674 with more information from his work. He sent along some glass tubes so that the "Curious Gentlemen may share my observations of Blood etc" (Leeuwenhoek, 1939b, p. 119). He also states that he has created "a more convenient Glass-pipe" for "seeing the motion of the Globuls in the Crystallin liquor of the Blood." He comments that he has sent Constantijn Huygens some tubes to forward to his son, Christiaan and that Christiaan has responded that he saw only "other particles," which Leeuwenhoek speculates might be the "red Globuls" that "come to stick to one another" and "exhibit odd and misshapen particles." Here again, Heringa suggests that some of the particles may be ***rouleaux*** of blood cells, which tend to form serpentine piles of cells. Leeuwenhoek's response is to Christiaan Huygens' vague comment, so Heringa may be creating an explanation that is not supported in the letter (Leeuwenhoek, 1939b, pp. 121–5).

On occasion, Leeuwenhoek veered into stunning metaphors to explain what he saw. In his 22 January 1675 letter to Oldenburg, he described.

> the globules in the blood as clearly as if we saw with our eyes without the help of glasses the grains of sand on a piece of black taffeta, some of them lying in a heap, others spread apart, and it is curious to see how the globules of the blood draw together when they lie scattered" (Leeuwenhoek, 1939b, p. 213).

"The grains of sand on a piece of black taffeta," was thought by Clifford Dobell and others to be an observation aided by some form of dark-ground or dark-field microscopy (Dobell, 1932, pp. 330–332). Additionally, Leeuwenhoek provided a beautiful metaphor drawn from the drapers' profession. Dobell extends his basis for the comment to include Leeuwenhoek's observations of "flagella and cilia and spirochaetes and micrococci with only a magnification of some 200–300x diameters" (Dobell, 1932, p. 331). A joint footnote by Schierbeek and Dr. Ir. Albert J. Kluyver, a microbiology professor at the Technical College of Delft, expands on Dobell's hypothesis by enumerating several additional microscopists who agree with Dobell and have developed methods for understanding what Leeuwenhoek might have been doing to achieve the dark-field effect (Leeuwenhoek, 1939b, p. 213).

Leeuwenhoek went into detail in his 26 March 1675 letter regarding serum color and the nature of blood. He observed that, while water is evaporating from the blood, "…in the clear substance, that was slightly yellow of colour, I could see nothing but some few particles that are not worth noticing and that looked like globules." Leeuwenhoek could not have centrifuged the "clear substance," so it may be that some of the globules were other particles that had avoided the clotting process. As Leeuwenhoek evaporated the substance, he saw a.

> very clear and transparent matter as if it had been glass except where it had been spread somewhat more thickly, where many peculiar figures could be seen, as if oblong crenulated leaves had sprung from one central point, each leaf having a peculiar shape with ribs and crenate sides, and from those leaves sprung other leaflets and all

those parts seemed to be composed of globules (Leeuwenhoek, 1939b, pp. 281–285).

Dr. Heringa comments that this set of observations is “far from clear” but attempts to unravel what Leeuwenhoek has reported. Heringa supposes that the globules or particles Leeuwenhoek witnessed are typical in serum that has gone through a thickening, evaporating, or inspissating[1] process. Leeuwenhoek’s poetic invocation of crenulated leaves with ribs and crenate sides Heringa puts down to the growth of “linked crystals forming along the fissures which soon appear in the above-mentioned film.”

In his last blood-related letter of 1675, Leeuwenhoek again made remarkable observations to Oldenburg. He amended comments from the previous letter that he had not seen “plain globuls move in that waterish matter without any evaporation made” in the serum, although there “were but very few of them.” The core of this letter’s blood observations lies with Leeuwenhoek’s sense that his blood cells are “firmer and harder” when he is ill than when he is well, as he was at the time of this letter. He finds the “…globuls of my Blood softer, and more sticking to one another, and my Body in a good state of health.” Dr. G. van Rijnberk, a physiology professor at the Municipal University of Amsterdam, comments in the footnotes that this is “once more a remarkable observation,” as it alludes to the tendency of red blood cells to “agglutinate more easily in health than in illness” and goes on to praise Leeuwenhoek for noting this, as one might have deduced the opposite. Leeuwenhoek wondered whether the illness might be caused by the changes from soft corpuscles to hard. Leeuwenhoek also reasons that the.

sanguineous globuls in a healthy Body must be very flexible and pliant, [if they shall pass through the very small veins (which as I have said, are in the films where the flesh is as it were inwoven, and through which the blood circulates, that is to say passes from the arteries into the veins); and that, in their passage, they change into an oval figure, reassuming their former globosity when they come into a larger room, in accordance with their size, this being owing to their strong and frequent movement] (Leeuwenhoek, 1939b, p. 301).

Dr. van Rijnberk adds a comment regarding the hardness of blood corpuscles as being “interesting in itself” and knows of no work that had assessed the relative hardness of blood cells and any impact that property might have on circulation through the arterioles, capillaries, venules, and veins. He also places Leeuwenhoek’s comment in the historical context of iatromechanics, influential in the 17th century (Musitelli and Bertozzi, 2018, p. 113). Iatromechanics suggested that the muscles, organs, and body all resembled machines; Leeuwenhoek’s ruminations about the hardness or pliability of corpuscles might have been influenced by this thinking.

Two years later, Leeuwenhoek found a way to focus past the stream of blood he had witnessed in the digestive processes of a louse and observe the “red globules” or red blood cells in “crystalline” fluid. His initial observations may have obscured a more cogent set of observations, or he may have been organizing his thoughts about what he had seen. In either case, he continued his work.

In a 14 May 1677 letter to Oldenburg, Leeuwenhoek made observations of brain tissue in which he differentiated between the “Brain-globuls, especially by the perfect roundness which the blood-globuls [have].” He confirms that the cells individually have “little or no colour,” but also says that the “sanguineous vessels, which run through the Brain” have blood that “in these small veins was yet red” (Leeuwenhoek, 1941, pp. 221–3).

In a footnote, Heringa writes that “…L. observed haemolysis in vitro, i.e. the phenomenon that the colouring matter leaves the blood-corpuscles so that the blood becomes transparent” (Leeuwenhoek, 1941, p. 225). This interpretation is difficult to support based on the English translation but may be more evident in the original Dutch.

In his 5 October 1677 letter, Leeuwenhoek explores a common, although spurious, belief about eel blood. He attempts to understand why the eel blood, when it gets in a fisherman’s eye, “causes an incredible pain lasting a whole day.” He observes that the blood cells are “thin rods about twice as long as the thickness of a blood-globule,” which Schierbeek explains is because Leeuwenhoek observed the blood corpuscles “on their narrow side,” rather than as round globules. Schierbeek notes that Leeuwenhoek would not recognize their lenticular nature until letters in 1682 and 1683 (Leeuwenhoek, 1941, p. 243).

In his 14 January 1678 letter, Leeuwenhoek wrote to Robert Hooke, author of *Micrographia* (1665) and then secretary to the Royal Society after Oldenburg’s death in 1677. Hooke had written Leeuwenhoek on 30 November 1677, but the letter had not arrived until 8 January 1678. Leeuwenhoek started the letter by writing that he had extended his consideration of blood globules from eels to his blood and concludes that he “doubts” the source of the eye pain is caused by eel blood. A blood-borne toxin causes the pain, although this would not be known until much later. Leeuwenhoek engaged in some new techniques to assess the pliability of the globules as he “…bended these Globules before my eyes, that they were three times as long as broad, without breaking [the vesicles which form the surface of the globules],” while “they recovered their former globulosity” (Leeuwenhoek, 1941, p. 307). Heringa adds that the shapes the blood cells take on may refer to “the usual disc shape (when seen on their flat side),” “the form of rods (when seen in profile),” or their biconcave shape “in the form of cups.” Leeuwenhoek did not mention this shape among the “many sorts of figures” alluded to in his letter (Leeuwenhoek, 1941, p. 307).

More notably, Leeuwenhoek then described the shape blood cells adopt while dehydrating. Each blood cell took on a thorny or rocky appearance while the globular, rod, or lenticular shape, depending on the viewing angle, was transformed while leaving the cell intact. Heringa calls these the “thorn-apple” formations. This cell shape can be formed in hypertonic salt solutions. Leeuwenhoek’s observation began a series of observations on the “smaller globules” that appear during dehydration; in this case, he saw “six smaller Globules.” If nothing else, his comments addressed the resolving power of his lenses, as the more minor deformations in the healthy blood cell must be approximately 1 μm or less in size (Fig. 1) (Leeuwenhoek, 1941, p. 309).

Interestingly, Leeuwenhoek described putting “…the greater Globules into so violent a motion, that their Vesicles burst into pieces, and then the lesser Globules appeared plainly to be scattered” (Leeuwenhoek, 1941, p. 311). This description clearly described hemolysis, while the previously cited Heringa passage is vague.

In a letter addressed to Hooke and dated only “March 1682,”

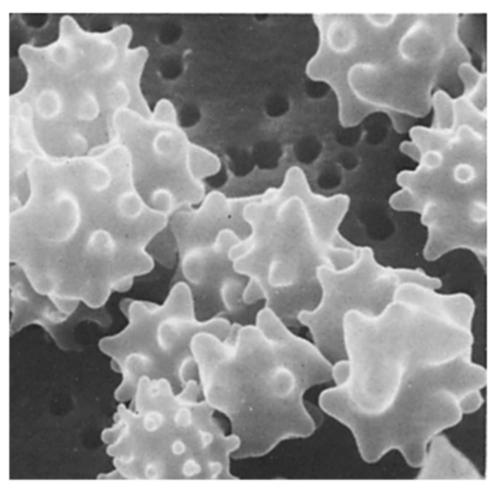

Fig. 1. Electron photomicrograph of erythrocytes in hypertonic solution, an example of the "thorn-apple" configuration of red blood cells (Sheetz et al., 1976, p. 196).

[1] Inspissating: to make thick or thicker due to evaporation or boiling.

Fig. 2. Erythrocytes in a fish's blood. (Leeuwenhoek, 1722, p. 51)(Leeuwenhoek, 1722, p. 51).

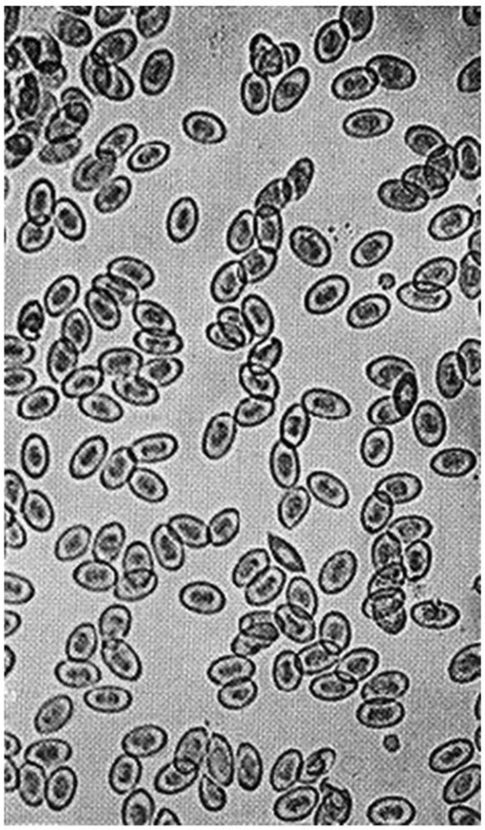

Fig. 3. Erythrocytes of a hen (Leeuwenhoek, 1952: 405).

Fig. 4. Agglutinated red blood cells as illustrated in Leeuwenhoek's Arcana Naturae Detecta (Haden 1939: 42).

Leeuwenhoek seems to have realized that blood globules are "flat, oval particles, thickish, floating in a crystalline water." He also repeated his previous observations regarding their individual and group color ("no colour" and "red colour") (Leeuwenhoek, 1948, p. 405). As these observations are made from the blood of cod and salmon, Leeuwenhoek noted something he has never seen in human blood: "…some of them enclosed in a small space a little, round body or globule," which Heringa believes to be "the first observation of the nucleus which L. discovered in the erythrocytes of fishes." Heringa also notes Leeuwenhoek's comment that "…3, 4, 5, 6, nay as many as 8 globules" is probably due to heating of the blood sample, which causes the formation of vacuoles around the nucleus (Leeuwenhoek, 1948, p. 407).

In a 22 January 1683 letter to Christopher Wren, who had concluded his tenure as president of the Royal Society in 1682, Leeuwenhoek made some minor comments regarding the effect of mixing his blood with *sal volatile oleosum.* He was interested in comments by an unnamed physician that "many people suffering from fever were entirely cured after having taken *Sal volatile oleosum*." The archaic medication was a mixture of cinnamon, nutmeg, nutmeg flower, lemon tree bark, salts of tartrate and ammonium, and alcohol, with some fermentation and distillation steps included in its preparation. The ratio of the ingredients probably varied from one apothecary to another, but it was supposed to "make the blood very quick and thin." It is interesting to note that Dr. Pieter van der Wielen, formerly a professor of pharmacology at the Municipal University of Amsterdam, states in the footnotes that *sal volatile oleosum* was probably used as a carminative or a medication that expelled gas from the stomach and bowel. Dr. van der Wielen indicates that around 1952, *Eau des Carmes,* an herbal preparation, or *Spiritus polyaromaticus,* a distillate preparation from balsam, both archaic remedies in their own right, could be substituted for *sal volatile oleosum* (Leeuwenhoek, 1952, p. 29). Heringa comments that the effect that Leeuwenhoek witnessed when mixing an equal or double amount of blood with *sal volatile oleosum* was probably hemolysis of the blood cells. Leeuwenhoek also speculates on a correlation between his blood's appearance and the illness he was suffering. This belief originated in the Middle Ages and continued to affect thinking in the 17th Century (Leeuwenhoek, 1952, p. 31)(Leeuwenhoek, 1952, p. 31).

He wrote Wren again on 16 July 1683, although it is unclear why Leeuwenhoek still wrote to Wren as he had ceded the presidency to John Hoskyns, another founding member of the Society. In this letter, Leeuwenhoek extended his comments regarding the colors of blood corpuscles to include those of the frog but confirms what he has "previously said about the salmon etc" (Fig. 5). Otherwise, Leeuwenhoek describes "a very light oval-shaped glimmer at the center" of some globules, "while others seemed to be composed of many oval disks, some larger than the others." Heringa suggests (1) that the glimmer was due to the nucleus shimmering "…when the microscope is adjusted to a deep level. If the adjustment is outside the level of the centre of the blood-corpuscle diffraction lines arise, parallel to the circumference, which causes L. to speak of 'many oval disks, one larger than the other,'" and (2) that the "very small globules" are "in all probability particles of broken up thrombocytes," which are the nucleated cells found in amphibians instead of platelets. The thrombocytes aggregate to form clots, much as platelets would in mammalian blood (Leeuwenhoek, 1952, pp. 73–5).

On 14 April 1684, Leeuwenhoek wrote Francis Aston, secretary of the Royal Society from 1681 to 1685, to confirm that there is "no difference in size between the globules in the blood of human beings and the said [ox, sheep, rabbits] animals," and that the blood of fishes, birds, and "all animals that live in water, consists of no other figures than flat,

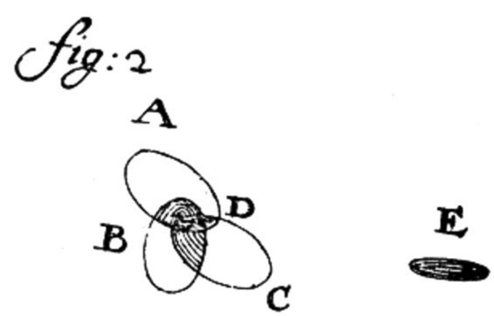

Fig. 5. Erythrocytes of a frog (Leeuwenhoek, 1952, p. 390).

oval particles" (Fig. 3) (Leeuwenhoek, 1952, pp. 241–3).

Leeuwenhoek's subsequent twelve letters — from 24 July 1684–12 August 1692 — including content relating to blood globules are all addressed to "The Royal Society," rather than to a specific secretary or president of the Society. Leeuwenhoek's 24 July 1684 letter provides an alternative estimate of the size of a red blood cell, previously estimated in his 1 June 1674 letter:

> I determined that the perfect globules, which colour our blood red, are of such size that if 100 of them were put end to end they would not attain the diameter of a coarse grain of sand, so that a 1.000.000 [1 million] blood-globules are the same size as one coarse grain of sand" (Leeuwenhoek, 1952, p. 267).

His 25 May 1688 letter, after a nearly four-year hiatus from describing blood cells, describes mixing oil and water in various proportions with substances, including blood. He seems to revert to describing the globules as having "complete roundness." Given his description of the water and blood mixture, it may be that Leeuwenhoek saw erythrocytes in a hypotonic solution, a condition that would cause the blood cells to take on water and lose their lenticular characteristics. This observation was accompanied by statements that "no globules whatsoever were cohering together, but that each lay separate from the others (which I had never observed)." He went on to say that "nearly all the globules of blood were composed of several globules, which was a very agreeable sight for me, especially when I made such a large multitude of completely round globules, all of the same size, move about together." This statement remains mysterious, although it may be that he was witnessing the result of blood in hypotonic conditions in the process of hemolysis. The editors do not mention the possibility of hypotonicity, nor does hemolysis of the blood cells lead to "nearly all the globules of blood" being composed of "several globules" (Leeuwenhoek, 1964, pp. 191–3).

Later in the same letter, Leeuwenhoek experimented with adding salt derived from bladder stones to blood and seeing the opposite effect — hypertonic, high salt solutions cause the aforementioned thorn-apple appearance in red blood cells, as commented by the editors, although Leeuwenhoek also said that the appearance of the globules is "…as if the heat had expelled, or driven out, the moisture from them." He further characterized the possibly hypertonic cells:

> For each globule of blood almost showed its particular shape; some were flattish, and one could see that they, in their turn, consisted of globules; others resembled dead little animals with legs: in short, I saw so many figures that one can not give them names (Leeuwenhoek, 1964, p. 203).

Leeuwenhoek might have seen the result of an inhomogeneous salt solution diffusing through the blood cells, resulting in various forms of blood cell; this would probably not occur if the solution were homogeneous. In the next paragraph, he described a phenomenon similar to hemolysis, although he described what he saw and does not categorize the phenomenon:

> After this I took the water in which the volatile salt had melted, this water I also mixed with blood, and at once observed that many globules of blood changed or fell apart in such a way that the globules could no longer be recognized, except by giving the closest attention, whereas other globules of blood remained whole for a long time. But this I did not observe unless I had mixed very little blood with much water (Leeuwenhoek, 1964, pp. 203–5).

Leeuwenhoek had become fond of the experiments combining blood, oil, and water with and without salt from accreted stones such as the bladder stones and *lapis bezoar*. When he mixed blood with oil, the blood cells "…at once coagulated together so firmly as I had never seen before, and however much I moved the mixture about, the globules of blood invariably stuck together, although I had left the same lying for a whole night". The editors believe that Leeuwenhoek witnessed agglutination here, wherein the cells clump together as their surfaces are more attracted to each other than to the oil in which they are suspended. In further experiments with a solution of oil, water, and a volatile salt, he again witnessed the formation of the irregular shapes characteristic of blood in a hypertonic medium. These "burst apart, as it were, although they still clung together by their surfaces, and these again to other globules." He observed this process while seeing that "these globules of blood became fewer and fewer, and that the water began to take on a red colour" (Leeuwenhoek, 1964, p. 247). Leeuwenhoek followed these experiments with additional ones that produced hemolysis (Fig. 4) (Leeuwenhoek, 1964, p. 257).

In his 7 September 1688 letter to the Society, Leeuwenhoek makes some observations regarding the movement of blood globules in tadpoles ("frog-worm"). In some ways, he simply confirmed previous statements, that "Arteries were not wider than to allow a single particle of blood (which, so to see, looked like globules, while they are nevertheless flat, oval particles," but he also….

> …saw that the particles of blood, because of the thinness of the blood-vessel, changed into a long, round shape, and when I took the little Animal out of the water, and it got so far that it began to die, I saw that the blood in the thinnest Arteries sometimes stood still, and when the blood in the same Vessel was again being driven forward, I saw that several particles of blood became stretched quite twice as long as the width of such a particle, and that they then appeared to taper to a point at both ends (Fig. 5) (Leeuwenhoek, 1967, p. 29).

While Leeuwenhoek's numerous comments on blood circulation in various creatures lies beyond the scope of this paper, he also witnessed "Arteries so long as they carry the blood into the furthest parts of the small vessels; and Veins, when they carry the blood back to the Heart" (Leeuwenhoek, 1967, p. 29). The editors state that this and other comments Leeuwenhoek makes in his letters describe the anastomosis of arteries to veins and confirms Malpighi's discovery, although Leeuwenhoek would have been unaware of Malpighi's work (Fig. 6) (Robertson et al., 2016, pp. 139–40).

Observations from letters written on 7 September 1688 and 12 January 1689 principally restate earlier observations regarding the size and shape of blood globules. However, a footnote for the 7 September letter suggests that Leeuwenhoek extended his observation to frog red blood cells and that the cell diameter would be about 9 μm. This size is consistent with what we know currently about frog red blood cells.

One aspect of Leeuwenhoek's observations that makes them so valuable to the history of science is that they are observations of dynamic processes. He not only sees a globule, round from one perspective and flat from another, but follows its progress as it moves through the blood medium, through the arteries, capillaries, and veins, as the globules are stretched into long, thin cells on their way through capillary beds, or as they tumble:

> Now the fact that these particles appeared to me in so many different changes of shape, was because these parts many times turned over (so to speak) in their course: For that which I, at one moment, was lying on one side before my eyes, lay after a little progress with its flat side before them: again, another particle of blood was thrown about in its length within the progress of a hair's breadth. In short, I here saw as many overturnings of the flat particles of blood as I could possibly imagine (Leeuwenhoek, 1967, p. 53).

Later in the 12 January letter, Leeuwenhoek started a series of observations in shrimp and crab — arthropods that do not have red blood cells or hemoglobin — although again Leeuwenhoek simply accounts for what he sees: "These globules had no colour and were somewhat transparent" (Leeuwenhoek, 1967, p. 111). Leeuwenhoek observed hemolymph and hemocytes, the "blood" and oxygen-carrying fluid in all arthropods – insects, crustaceans, spiders, and many other classes. We

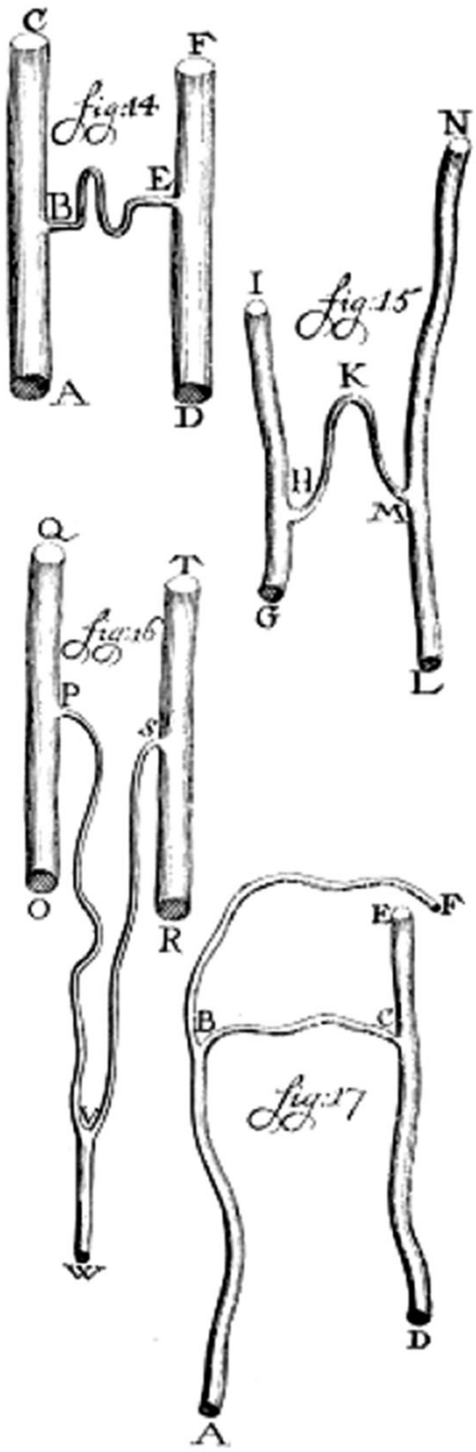

Fig. 6. Figures 14, 15, 16, and 17 show the junction of artery and vein in perch, pike roach, and carp (Leeuwenhoek, 1967, p. 391).

now know that the hemocytes carry hemocyanin, a copper-containing protein that facilitates oxygen transport in arthropods. Just as Leeuwenhoek would not have known about hemoglobin, he would not have known about hemocyanin.

His 1 April 1689 letter contains a lengthy set of observations of blood circulation in a bat, which he keeps in a box and feeds for several days. Leeuwenhoek was particularly interested in the nature of congealed or clotted blood in the ears and wings but the letter does not contain any new information regarding the blood cells (Leeuwenhoek, 1967, pp. 159–169).

In his letter of 24 June 1692, Leeuwenhoek observed the hemolymph of a grasshopper. The "…blood that was in these vessels had a green Colour". The editors correct the notion that the grasshopper had green blood and state that the green coloration is due to chlorophyll circulating in the hemolymph. As is typical for him, Leeuwenhoek pushes his observation as far as possible – down to resolution of a single globule:

> But when the blood formed so thin a layer that I judged it to be only a single globule of blood, it had no colour whatsoever; and then it most nearly resembled a transparent substance. And here it was clear to me that all these green globules were embedded in a thin transparent liquid (Leeuwenhoek, 1976, pp. 51–3).

In his 12 August 1692 letter, Leeuwenhoek went a bit astray:

> For all the brilliant, beautiful red colour of Arterial blood, in contrast to the blood from the Veins, is only due to the fact that Arterial blood contains more of the substance which is called the Serum of the blood than does the blood in the Veins, and the less there is of the so-called serous substance in the blood, the more the blood tends towards black, as has also been said on a previous occasion (Leeuwenhoek, 1976, p. 121).

The idea that arterial blood is a "brilliant, beautiful red colour" due to the presence of more serum may have made sense from a colorimetric point of view, but Leeuwenhoek would not have known about the oxidation-reduction cycle of hemoglobin at the time he made his observations. The oxygen-binding properties would remain a mystery until scientists made a series of discoveries starting in the early 19th century. Still, his comment underlines his strength as an observer rather than a theoretician.

With his 14 September 1694 letter, Leeuwenhoek resumed writing to individuals within The Royal Society. Richard Waller, appointed secretary of the Society in 1691, received the third of Leeuwenhoek's letters commenting on arthropod "blood," this time with a focus on crab hemocytes. Leeuwenhoek's observation is remarkable for the dynamic portrait it presents of the hemocytes in circulation, but also because he commented that they are round globules "although they were not red," so his comment captured the truth of the matter rather than letting his previous statements bias him.

> And no sooner had I done this but I saw at once such an inconceivably large number of blood parts, which appeared as round globules to the eye, although they were not red, but only less transparent than the fluid in which they floated, flowing through the blood vessel we call a Vein, and that with such speed and in such large numbers that no Human Being can understand it unless he sees it with his own eyes. Nay, I can compare the said round blood parts moving past my eyes no better than as if we thought we saw through a large tear or the opening of a Window or the like a snowdrift propelled by a strong wind moving past our eye, and I do not know that I ever saw blood being propelled with such speed through its vessels (Leeuwenhoek, 1979, p. 149)(Leeuwenhoek, 1979, p. 149).

Leeuwenhoek's 10 April 1695 letter to Antoni Heinsius, then Grand Pensionary of the States of Holland and a friend of Leeuwenhoek's since the 1660 s (Anderson, n.d.), expanded on hemocyte observations:

> the Blood globules which were propelled in all the vessels are very few in number in comparison with the globules that are in the blood of the Animals living on the earth or the Animals or Fishes which are in the Waters and whose blood is red. Nay, I even believe that the blood globules of those which have red blood are at least twenty-five times more numerous than those I perceived in the crab (Leeuwenhoek, 1979, p. 171)(Leeuwenhoek, 1979, p. 171).

Dr. D. H. Spaargaren, a biologist, then with the Netherlands Institute for Sea Research at Den Hoorn, comments that there are about 50,000 hemocytes per mm^3 in crabs and 4.5–5 million per mm^3 in humans (this varies by gender; normal ranges also are somewhat broader than Dr. Spaargaren states), so Leeuwenhoek's estimate here is four times too low.

A 25 September 1699 letter, again to The Royal Society, made the incorrect observation that frog tadpole blood, once clotted, can resume its previous corpuscular form and flow again after a few days. In the footnotes, Dr. A. D. F. Addink, a professor of Animal Physiology at the State University of Leiden, corrects this by adding that phagocytes deconstruct clots, an irreversible process discovered a few centuries afterward (Leeuwenhoek, 1989, pp. 343–7).

Leeuwenhoek wrote Hans Sloane, secretary of The Royal Society since 1693, a letter dated 9 July 1700 that makes some minor comments about blood cells in flounder. However, he provides details about blood vessels' size compared to the corpuscles, thus clarifying some circulatory questions in the flounder (Leeuwenhoek, 1993, pp. 135–7).

Further on in the same letter, Leeuwenhoek provided comments on experiments involving higher magnification lenses. The effect of using the higher magnification was that the blood cells moved past the lens more swiftly. Leeuwenhoek then would pinch the blood vessel, which caused the blood "particles" to be "so far apart that no blood particles, not even those of which six had made up one blood particle, were to be seen in those vessels, but only a homogeneous fluid substance, which was slightly coloured, flowed through the vessels" (Leeuwenhoek, 1993, p. 147). He also confirmed the oval shape of the blood cells. Leeuwenhoek provided some drawings of his observations (Leeuwenhoek, 1993, p. 426). However, he also provided some new details, which remain a bit mysterious [n.b.: figure numbers in the following paragraph refer to Leeuwenhoek's numbering shown in Fig. 7]:

> I made up such a globule as shown in Fig. 5 out of six wax globules, so that I could show the blood globules to those who ask me for an exact description of these globules, adding that I am certain that each of our blood globules is composed at least of thirty-six globules. When the globules so composed are squeezed and moved, they are compressed because they are flexible, and they assume a perfectly spherical form, as shown in Fig. 6. It is easy to understand that through this disposition the globules of our blood and of that of animals acquire their roundness, but we cannot conceive how the said oval blood particles are composed out of six globules (Fig. 7) (Leeuwenhoek, 1993, pp. 147–9; 426).

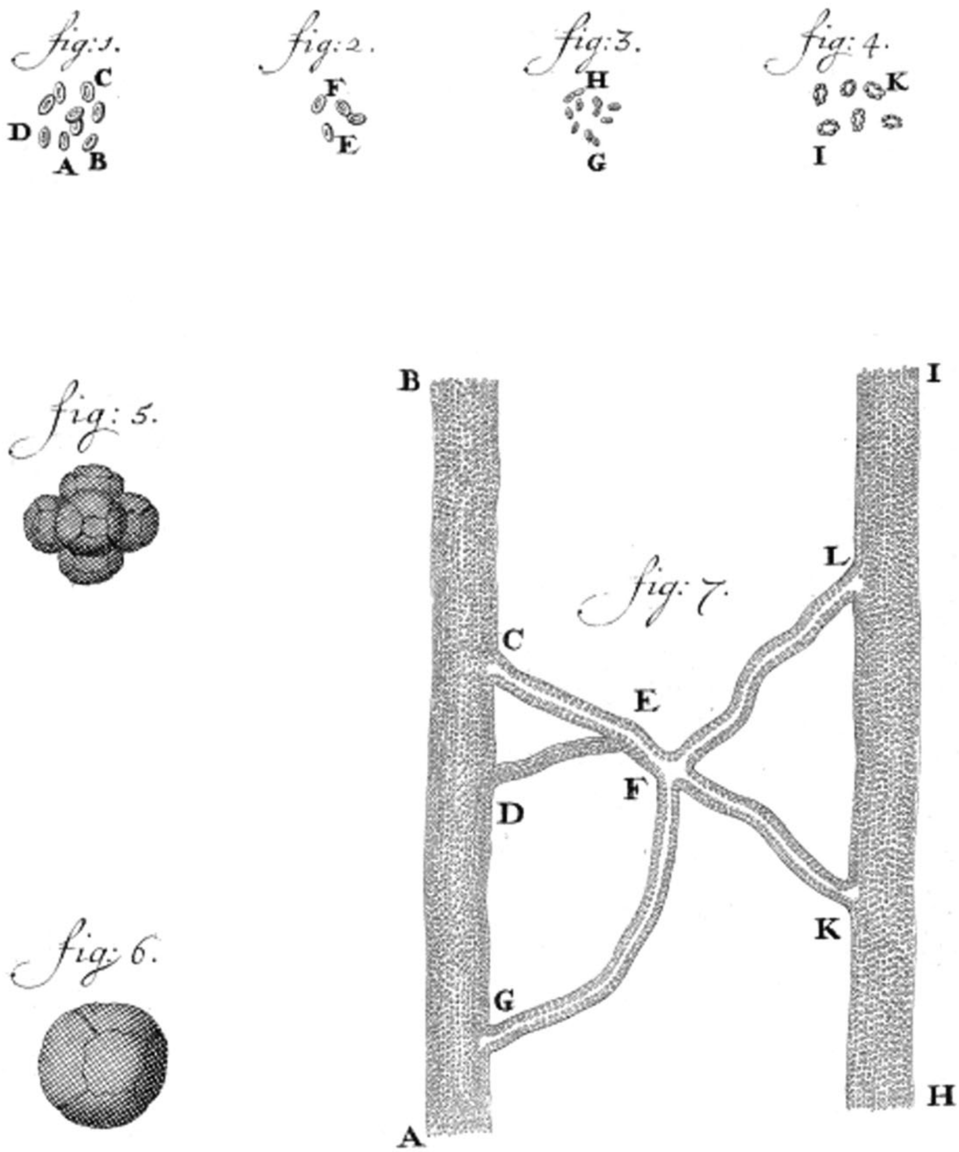

Fig. 7. Erythrocytes. Fig. 1 salmon; Fig. 2, Fig. 3, Fig. 4 flounder; Figs. 5, 6 wax models of the structure of erythrocytes, according to Leeuwenhoek (Leeuwenhoek, 1993: 426).

It may be that the flounder erythrocytes, which are nucleate, show divisions into six subunits, but it remains unclear to what Leeuwenhoek was referring to when he wrote about thirty-six globules. He further described a vision of the microscopic realm that is very perceptive, although a bit exaggerated:

> I learned to my astonishment that there are some people who venture to say that they will describe the origin of some things. For my part, even if I could discover the form of particles a thousand million [1 billion] times smaller than a blood globule, I am certain that I should still be far from being able to see their first constituents (Leeuwenhoek, 1993, p. 149).

If Leeuwenhoek could have seen 1000 times smaller, he could have seen antibodies (~15 nanometers in diameter); if 10,000 times smaller, he could have seen individual water molecules (280 picometers) and carbon atoms; 1 billion times smaller (~7 femtometers) would have allowed him to view a proton – beyond the resolution power of light microscopy. His comment revealed that he was not entirely content with what he saw but wanted to see even further into life's tiniest of constructs.

In a 21 June 1701 letter to The Royal Society, Leeuwenhoek added to previous comments on spider blood hemocytes. As he has done in previous notes on hemocytes, he dismisses the "ancient" idea that the spider and other arthropods were bloodless animals. Leeuwenhoek managed to find poetry in the moment:

> Although this animal will have been known among the ancients as a bloodless animal because they have not seen any blood-red substance when they broke up these little animals or killed them, still I saw the particles of blood, which to all appearances were round globules, propelled in a liquid substance, so clearly as if one saw small peas rolling down a slanting board (Leeuwenhoek, 1993, p. 321).

In a letter to Heinsius dated 17 December 1712, the last of the published letters to mention blood corpuscles, Leeuwenhoek returned his attention to shrimp blood, at first confirming what he had reported previously, then commenting on the salts that formed after the hemolymph had been allowed to evaporate:

> after I had cut the shrimp crosswise I found that as much as a drop of blood emerged from the fish-parts, which I observed, and I saw again the transparent globules of blood which floated in the watery fluid, the serum. After these blood-globules had been outside the shrimp for some little time, they floated in the fluid together in greater or smaller numbers, without coagulating, and only touching one another (Leeuwenhoek, 1993, p. 23).

With this comment, Leeuwenhoek ended his observations of blood globules, red and transparent, although throughout Volume 17 he continued to add to his notes on circulation. Strangely, his first and last observations of blood were in arthropods. His comments on a louse feeding on blood in 1673 had not noted the hemocytes he would discover in shrimp about sixteen years later.

4. Conclusion

Recognition of the enormity of his work, which added nuance and detail to revelations provided in earlier letters to his correspondents, has been less than complete. Although this may have been due to incomplete editions of his letters before the publication of *Alle de Brieven*, the recognition has remained slight.

Why are Leeuwenhoek's many observations of blood cells important? Given his consuming curiosity and the resolution of his microscopes, it may have been inevitable that Leeuwenhoek became the first scientist to assess the number of red blood cells to fit in a volume (e.g., "250000 times smaller than a grain of sand" (Leeuwenhoek, 1939a, p. 103) or e.g., "1.000.000 blood-globules are the same size as one coarse grain of sand" (Leeuwenhoek, 1952, p. 267)). He was a keen observer of corpuscle behavior under various conditions (e.g., dehydration of the serum and blood, hemolysis, shape distortion when passing through capillaries). Leeuwenhoek also commented on the absence of color in blood cells when their "thickness [is] not amounting to more than four or five globules" (i.e., cells) (Leeuwenhoek, 1939a, p. 85). Later letters described the blood changing from red to "dark-red or blackish"(Leeuwenhoek, 1939a, p. 95). In addition, he noted that whatever was circulating in arthropods was not the same as his "red blood globules." His observations often are lumped together with those of Marcello Malpighi and Jan Swammerdam, although their observations were brief and incomplete, whereas Leeuwenhoek's observations spanned much of his life.

In a sense, his single-lens examination of red blood cells could be considered a prelude to the hemocytometer, a lens-based device that would go through several iterations in the mid-19th century. While his observations were far from foreshadowing the invention of hemoglobinometers — colorimeters measuring hemoglobin concentration — his work might have alerted scientific minds in the 18th century that assessing color could be important for evaluating the properties of blood in general and red blood cells specifically.

Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micron.2022.103249.

References

Anderson, D., n.d., Lens on Leeuwenhoek [WWW Document]. Anthonie Heinsius. URL ⟨https://lensonleeuwenhoek.net/content/anthonie-heinsius⟩ (accessed 11.5.21).
Boyle, R., 2000. Memoirs for the Natural History of Human Blood. In: Davis, M.H.E.B. (Ed.), The Works of Robert Boyle: Notion of Nature and Other Publications of 1684-6, Volume 10. Pickering and Chatto, London, pp. 3–102. Notion of Nature and Other Publications of 1684-6.
Cole, F.J., 1937. Leeuwenhoek's zoological researches.—Part I. Ann. Sci. 2, 1–46. https://doi.org/10.1080/00033793700200391.
Dobell, C., 1932. Antony van Leeuwenhoek and his "Little Animals.". Harcourt, Brace and Company,, New York.
James M. Stengle, P.M.N., 1974. Historical Introduction. In: Surgenor, D.MacN. (Ed.), The Red Blood Cell. Academic Press, New York.
Leeuwenhoek, A., 1993. Alle de Brieven. Deel 13: 1700-1701. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1989. Alle de Brieven. Deel 12: 1696-1699. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1979. Alle de Brieven. Deel 10: 1694-1695. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1976. Alle de Brieven. Deel 9: 1692-1694. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1967. Alle de Brieven. Deel 8: 1688-1692. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1964. Alle de Brieven. Deel 7: 1687-1688. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1952. Alle de Brieven. Deel 4: 1683-1684. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1948. Alle de Brieven. Deel 3: 1679-1683. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1941. Alle de Brieven. Deel 2: 1676-1679. N.V. Swets & Zeitlinger,, Amsterdam.

Leeuwenhoek, A., 1939a. Alle de brieven. Deel 1: 1673-1676. Taylor & Francis,, Amsterdam.
Leeuwenhoek, A., 1939b. Alle de brieven. Deel 1: 1673-1676. N.V. Swets & Zeitlinger,, Amsterdam.
Leeuwenhoek, A., 1722. Opera Omnia Seu Arcana Naturae. Joh: Arnold Langerak,, Leiden.
Musitelli, S., Bertozzi, M.A., 2018. A brief historical survey of the anatomophysiology of the human body. Arch. Sci. 2, 113.
Robertson, L., Backer, J., Biemans, C., van Doorn, J., Krab, K., Reijnders, W., Smit, H., Willemsen, P., 2016. 8 the circulation of blood. In: Antoni van Leeuwenhoek. Brill, Leiden, pp. 136–148. https://doi.org/10.1163/9789004304307_009.
Robinson, J.P., 2013. Wallace H. Coulter: Decades of invention and discovery. Cytom. Part A 83 A 424–438. https://doi.org/10.1002/cyto.a.22296.
Sheetz, M.P., Painter, R.G., Singer, S.J., 1976. Biological membranes as bilayer couples. III. Compensatory shape changes induced in membranes. J. Cell Biol. 70, 193–203. https://doi.org/10.1083/jcb.70.1.193.
Stein, J.B., 1931. On the Trail of Van Leeuwenhoek. Sci. Mon. 32, 116–134.
Wintrobe, M.M., 1985. Hematology, The Blossoming of a Science. Lea & Febiger,, Philadelphia.
Wintrobe, M.M., 1980. Blood, Pure and Eloquent. McGraw-Hill Book Company,, New York.