Review Article

Biological Significance of the Erythrocyte Sedimentation Rate (ESR) Test: Pandemic Reemergence of Robin Fåhraeus's "Fibrin Coagula" – Historical Overview

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In the pre-antibiotic era, infections were usually more frequent and serious than today. Robin Fåhraeus (1888-1958) examined the erythrocyte sedimentation rate (ESR) test for infections, which was normally carried out *in vitro* with freshly drawn blood. His extensive studies on the mechanism and physiological significance of the enhanced sedimentation of erythrocyte aggregates (rouleaux) in disease included *in vivo* simulation. This led him to propose an explanation for the finding of long white strips ("fibrin coagula") within the blood vessels of those who had died from infections. The surge of serious infections in pandemic times has likely kindled a reemergence. He further speculated both that the weak aggregation of red blood cells (RBCs) followed the liberation of water molecules from their surfaces, and that the importance of their aggregation, which was induced by changes in serum proteins (not necessarily antibodies), extended beyond the clinic. In modern times these changes have led to immunologically significant entropic interpretations of infection-associated aggregations, whether cellular (e.g., RBC) or molecular (i.e., macromolecular polymerizations). Thus, rouleaux formation displays a process at the cellular level that can proceed in parallel at a less visible macromolecular level. It has been proposed that, when intracellular, aggregations would discriminate between self and not-self proteins in the crowded cytosol. Favoured by an associated pyrexia, this could lead, by mechanisms to be determined, to the preferential loading of peptides from proteins deemed foreign for presentation as MHC complexes to specific clones of immune cells.

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1. Introduction – ESR High in Infections

As with the thermometer, subjecting human blood samples to the erythrocyte sedimentation rate (ESR) test provides a non-specific indicator of disease, especially infections. Often associated with immune responses (antibody formation, leukocyte increase), there are also changes in the concentrations of certain proteins (including "acute phase proteins"). This correlates with a loss of the "suspension stability" of individual red blood cells (RBCs; erythrocytes), which form aggregates ("piles of coins;" rouleaux) that settle rapidly in vitro.

The serum-dependent physicochemical nature of the phenomenon was indicated in 1908 with a report that normal serum could be made rouleaugenic merely by heating (60^{o}C, 15 min).[1] In the 1980s, this was shown to reflect the polymerization (specific aggregation of individual molecules) of serum albumin. Furthermore, normal serum can also be made rouleaugenic by slight concentration (lowered water content),[2] or by adding compounds such as polyethylene glycol that are also known to engender molecular-level aggregations.[3]

Rouleaux formation was deeply studied by the Swedish physician-scientist Robin Fåhraeus (1888-1968), whose doctoral thesis was published in 1921[4] and was expanded in his later works.[5][6][7] He noted that the ancient Greeks had regarded the serum change as a *cause*, rather than a *result*, of disease. Despite arguments to the contrary by Paracelcus (1493-1541), bloodletting to remove the white "phlegm" – deemed-toxic – had endured for millennia.

The present paper first considers the ancient postulate of four "humors" in body fluids, of which only phlegm was infection-specific (section 2). There then follows Fåhraeus's demonstration that the ESR test could be performed in vivo by arresting blood circulation in a vertical vein, so creating an upper white fibrous strip above the RBC sediment (section 3). He related this to the "fibrin coagula" reported in pre-antibiotic times. This likely explains similar autopsy observations made during the recent pandemic (section 4). The paper then turns to Fåhraeus's hypothesis that the aggregation of RBCs is associated mechanistically with the liberation of water molecules bound at their surfaces, which can now be understood in entropic terms (section 5). The paper concludes by exploring his view that the "great and usual blood change" manifest as aggregated RBCs in infected subjects[4] is of broad physiological importance, for which much evidence has since accumulated (section 6).[8][9][10][11][12]

2. Fåhraeus and the Four Humors

Having, like many of his contemporaries, received a classical education, Fåhraeus knew of the ancient Greek postulate of four humors, for three of which there were obvious associations with colors – “choleric” (yellow, as in jaundiced subjects), “sanguine” (red), and “melancholic” (black). The Greeks associated the fourth – white "phlegm" – with disease. To rid the body of phlegm, sick persons were bled.

Fåhraeus wondered how this practice might have arisen. He speculated that in ancient times ritual bloodletting would have used earthenware vessels. Here, the unobserved clots would have contracted, expressing serum. On emptying a vessel, the Greeks would first have poured off the yellow (choleric) serum and then have dislodged the clot. This would have been red (sanguine) in its oxygenated upper part and black (melancholic) in its anaerobic base. Thus, three of the humors (yellow, red, black) would be expressed in blood from a normal person. However, in the case of blood from a sick person, an extra white layer (“phlegm”) would have been seen above the red part of the clot (Fig. 1).

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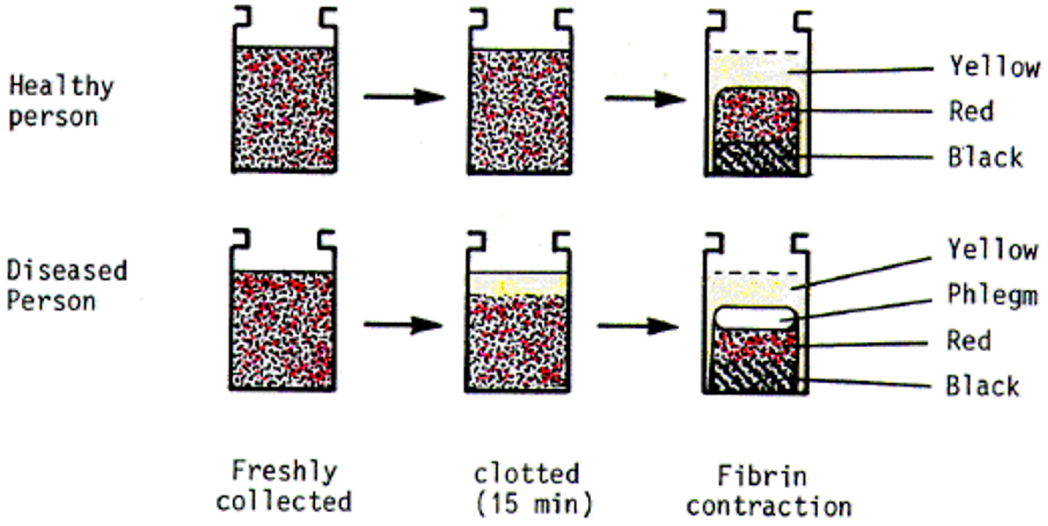

Figure 1. Generation of an additional layer (phlegm) in the contracted clot of blood from a diseased person. Blood from a healthy person (upper) or diseased person (lower), is either freshly collected (left), freshly clotted (middle), or left overnight for the clot to contract (right). Changes in plasma proteins in disease cause aggregation of red cells in blood. The RBC aggregates (rouleaux) sediment, *before* clotting can occur. This leaves a liquid upper plasma layer. The fibrin clot involves the *entire* volume of the sample. Subsequently, the clot contracts expressing yellow serum. A clot from a normal person shows two compartments, red (sanguine) and black (melancholic). An *extra layer* (phlegm), due to the clotted plasma, is seen in the clot of blood from a diseased person. Reproduced, with permission, from reference[10].

3. ESR Test In Vivo

Unlike antibody-mediated aggregation, the intercell linkage is weak (Fig. 2). Thus, stable rouleaux do not normally form in vivo in the flowing blood of a sick person. Typically, a column of freshly drawn blood is held stationary in vitro in a vertical glass tube. The length of the upper plasma layer that appears as the rouleaux settle is measured. Since blood clots rapidly when exposed to an external surface, it is citrated.

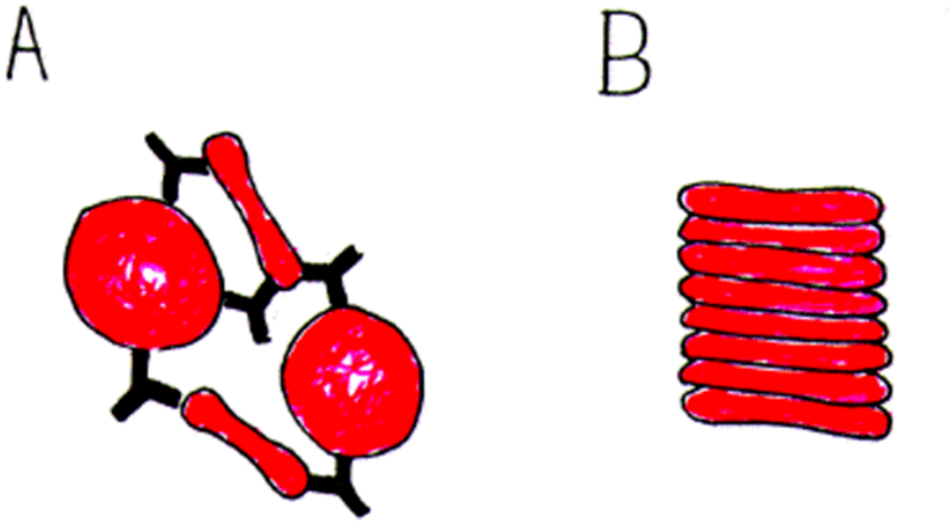

Figure 2. Distinction between (A) aggregation of red blood cells by an extracellular ligand (agglutinating antibody) and (B) aggregation which does not involve a cross-linking agent. In (A) the biconcave disk-shaped red cells are cross-linked by Y-shaped bivalent antibodies (not drawn to scale). In (B) the red cells adopt the energetically most favourable pile-of-coins conformation (rouleaux), which sediment rapidly. Reproduced with permission from reference[10].

Fåhraeus predicted that the phenomenon would be observed in vivo if the blood flow were slowed (perhaps in an aneurysm or during a hypotensive episode) or if the heart stopped pumping (as after death). Furthermore, being in vivo, in the short term no anticoagulant would be required. His simple experiment with two tourniquets is shown in Figure 3. After the experiment the tourniquets were quickly removed. Had this not been done, there would be the possibility of eventual intravascular clotting, so generating a long fibrous white upper strip above the red erythrocyte sediment below.

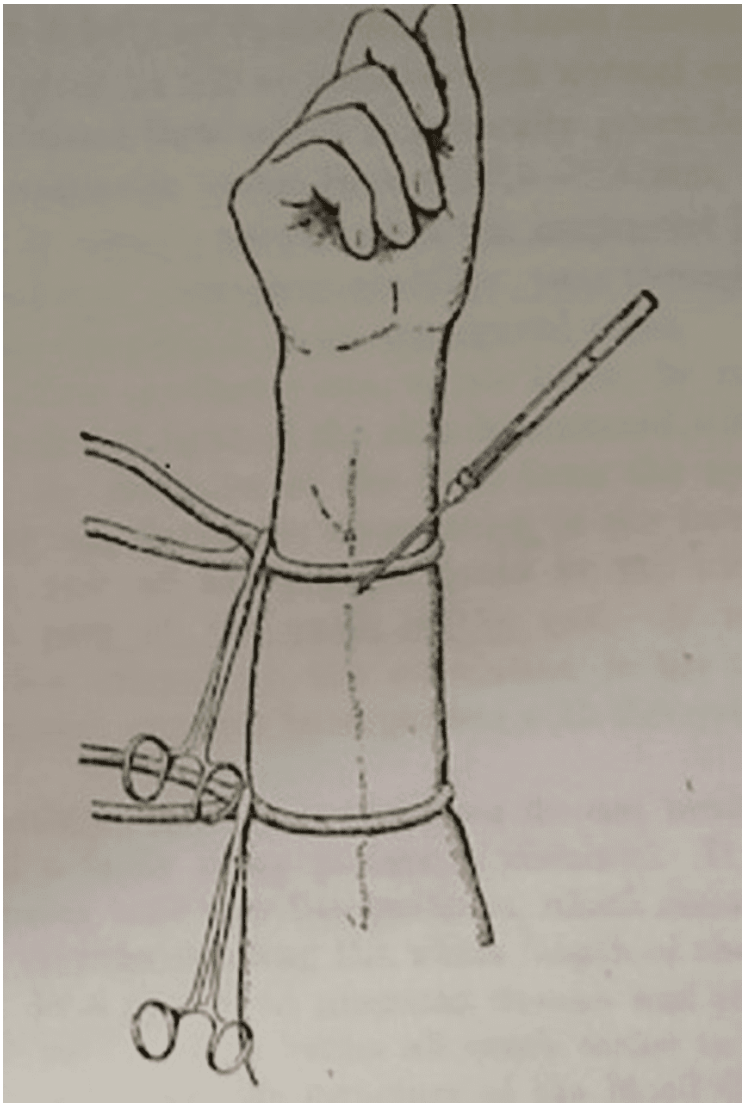

Figure 3. The ESR test in vivo. A sick person rests their elbow on a table with their forearm elevated. Fåhraeus ties two rubber tourniquets round the forearm, one low and one high. The high one, near the hand, stops the venous return and the lower one near the elbow prevents the blood draining out of the vein. In other words, he is simulating, in vivo, the ESR test normally carried out with a vertical glass tube. He has occluded a forearm vein, so the blood is not agitated as when it is circulating. Shortly thereafter, he puts a needle into the top of the occluded vein. Out comes pure plasma, free of red blood cells. With a healthy subject, rouleaux *do not form*, and he then withdraws red blood (plasma plus red blood cells). Reproduced from chapter 6 of reference 4.

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Fåhraeus extensively cited the early literature[13], which included slowing the blood flow in the back of the eye and watching the red cells attempting, but not succeeding, in forming stable rouleaux[7]. Thus, rouleaux formation would likely begin in vivo *within minutes* of the death of a someone undergoing an immune response to foreign antigen. Much later, in vivo clotting would occur. Indeed, in pre-antibiotic times, when *severe infections* were both *more common and more serious*, autopsies frequently revealed long white fibrous intravascular clots ("fibrin coagula," that would correspond to the erythrocyte-free plasma). Thus, Fåhraeus noted:[5] "Having had the opportunity ... of making autopsies on persons in whom the suspension stability [ESR test] was measured shortly before death, I may emphasize that strongly developed fibrin coagula are found only in cases which had previously a great sinking velocity" (i.e., high ESR).

4. Pandemics

By definition, in pandemics infections are also common and can be more serious. Indeed, ESRs are increased in those with serious COVID-19 infections[14][15]. So fibrin coagula would be expected at autopsies. During the recent pandemic there was concern about unaccounted "excess deaths"[16][17], some of which may have reflected both unrecognized cryptic factors that lowered blood pressure[18], and inadvertent vascular spread of intramuscular vaccinations[19], which generated SARS-CoV-2 spike protein[20]. This could interact with fibrin, so driving inflammatory blood clotting (thromboinflammation)[21][22].

Adding to such alarms, embalmers internationally observed white fibrous clots like the fibrin coagula. As reported to online interviewers and journalists, they were as mystified as were the physicians they consulted, who appeared unaware of the history. Fibrin clots in blood vessels were independently noticed in 2022 by pathologist Arne Burkardt and colleagues, who declared them "a mystery" in their autopsy reports, possibly related to novel forms of vaccination[23][24]. The first of many postings by a UK interviewer in 2024 garnered over a million visits in the first week[25].

5. Entropic Liberation of Bound Water

Although complex[26][27], among possible explanations for serum-induced changes in ESR values, Fåhraeus considered that "dehydration of the [red blood] corpuscle surface layer may be the predominant factor"[5]. At that time, the liberation of weakly bound water molecules from the surface

of macromolecules (such as albumin) was discussed in "entropic" terms mainly by chemists and physicists. Fåhraeus did not use the term in his work. However, studies of the aggregation of tobacco mosaic virus (TMV) coat proteins initiated in the 1930s have established its powerful influence in biochemical reactions, which include its increase with temperature[28].

As with the aggregation of *individual* RBCs, aggregation restricts *individual* macromolecules (e.g. proteins) by bringing them to order. Sometimes, this loss of entropy is driven by energy-yielding chemical reactions (exothermic). However, often aggregation is achieved at the expense of the water molecules that are trapped at protein surfaces. Raising the temperature tends to liberate these water molecules and the energy associated with this *disordering* of water more than compensates for the energy associated with the *ordering* of protein[29][30]. Referred to as entropy-driven aggregation, this confers on susceptible proteins an exquisite sensitivity to small increases in temperature that can occur within a narrow physiological range, permitting fine discriminations[10]. Indeed, consistent with this, in 1973 it was shown that over the temperature range corresponding to physiological pyrexia, rouleaux formation increased. A decrease above 41°C can be attributed to changes in cell structure leading to the generation of acanthocytes[31].

When added to normal blood, certain agents that aggregate proteins (e.g., polyethylene glycol PEG) [3] also aggregate RBCs[26]. In this respect, RBCs differ from large proteins only in that their aggregation can be easily observed microscopically. The self-aggregation of macromolecules (TMV, serum albumin) is referred to as their polymerization, although this term is not applied to aggregated cells. Yet there is specificity. Just as proteins can form individual like-with-like homoaggregates, so RBCs from different species, when mixed, form distinct rouleaux homoaggregates (Fig. 4)[8][32]. This potentially models the developmental sorting of cell types in embryos[33].

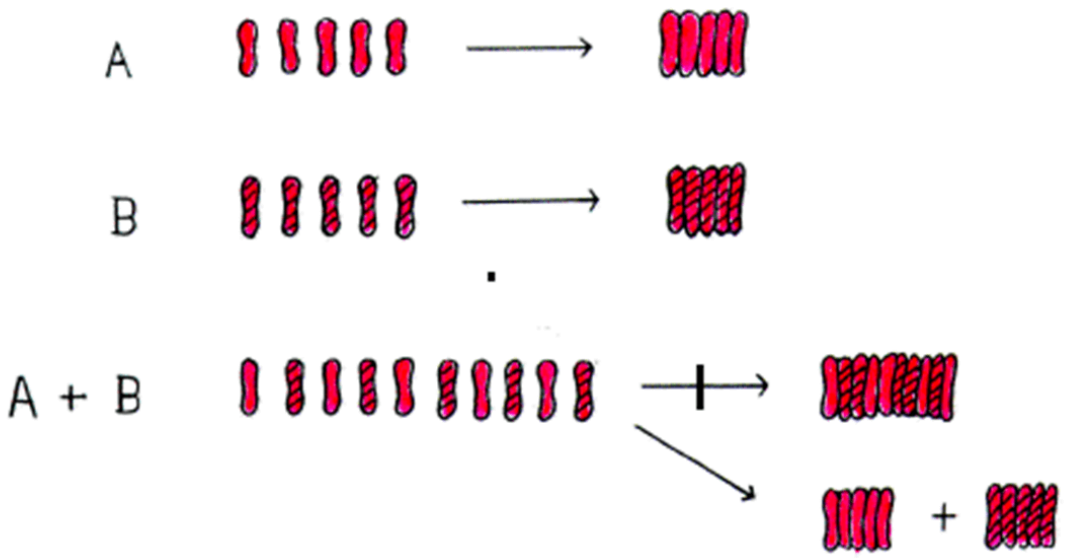

Figure 4. Self/non-self discrimination in rouleaux formation. When mixed, RBC populations from different species (A and B) self-assemble forming homoaggregates. Adapted from reference 10.

6. Broad Implications: The Aggregation Pressure Collective

In Fåhraeus's time, when blood antibodies had not been chemically characterized, their activities were often described by their functions (e.g., haemagglutinins, antitoxins). In Chapter 6 of his thesis he wrote: "A priori it appears probable that this apparently great and very usual blood change must be a change with important physiological and pathological consequences."[4] He suspected that the aggregation-promoting property of plasma exemplified a more broadly based phenomenon with important implications. More specifically he opined: "The plasma change ... might represent ... one phase of a protective reaction of the same kind as ... the numerous protective reactions which research of the last few decades has shown to take place in the blood. ... One of the most important protective reactions hitherto known, is the formation of antitoxin ... the formation of antibodies."[4] So: "Generally speaking, it may be said that a reduction of the suspension stability of the blood is one of the most common general reactions of the organism in disease, perhaps the most common. In this respect it may be best compared with such reactions as pyrexia and leukocytosis."[5]

Indeed, acting entropically, pyrexia would be expected to promote many molecular aggregations, both extracellularly and intracellularly. Building on prior ESR studies[2][8][9] we have considered the abilities of macromolecules or whole cells to retain or release water molecules from their surfaces in

terms of the "aggregation pressure" collective. Thus, depending on their relative concentrations, these elements can, to varying extents, both contribute to, and be acted on by, the total aggregation pressure[10]. This fine-tuning has provided insights into issues such as the evolution of sex chromosome dosage compensation, the female targeting of many autoimmune diseases, and genetic dominance[10][11][12][34] That such apparently disparate phenomena can be understand in the terms of one unifying principle is encouraging. We seem to have come a long way since the ancient Greeks.

Statements and Declarations

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Conflict of Interests

I declare no conflict of interest.

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Data Availability

As noted in legends, the figures are from prior works.

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Declarations

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