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EDITORIAL

ON RED BLOOD CELL (RBC) FILTERABILITY, PRESENTED IN 1958 BY ROBIN FÅHRAEUS, HIS CONTROVERSY TO KNISELY'S 'BLOOD SLUDGE' AND COPLEY'S CONCEPT ON BLOOD CELLULAR CLUMPING

Alfred L. Copley

Fåhraeus presented in 1958 at the 3rd International Congress on Rheology, held in Bad Oeynhausen, F.R. Germany from 23. - 30. September 1958, a paper on the aggregation of red blood cells (1).

The Proceedings of this Congress began publication in Volume 1 of Rheologica Acta, as double issue number 2/3, in 1958, but the contribution of Fahråeus, although published in Volume 1, appeared in 1961 after an interval of three years when publication of this journal was resumed by Dr. Dietrich Steinkopff Verlag, Darmstadt, F.R. Germany.

This contribution by Fåhraeus was to my knowledge never dealt with in the literature. Because of its importance I am bringing it to the attention of the Readers of our Journal, as it otherwise may remain unnoticed.

I was greatly surprised that in none of the contributions at the International Symposium on Filterability and Red Blood Cell Deformability held in Goteborg from 11. - 13. September 1980, it was referred to by any of the participants. This led to my mentioning it at the banquet of this conference. I heard later from several participants of this symposium, who are actively engaged in research on RBC filterability and deformability that they had no knowledge of this contribution by Fåhraeus. It is for this reason that I give a brief description of his test in the hope that it will be employed in future studies on RBC filterability either in the way Fåhraeus described it or in some suitable modification.

According to Fåhraeus (1), he developed the RBC filterability test in great part on the basis of his concept of the circulation enhancing property of rouleau formation of RBC in certain parts of the vascular system. In particular, he cited his work with T. Lindqvist (2) which is now known as the Fåhraeus-Lindqvist effect(3).

Fåhraeus designed an apparatus which consisted of a cylindrical brass container. It holds at its bottom a circular piece of glass. In this container six circular pieces of filter paper, 23 mm in diameter, are placed on top of each other and pressed down at their periphery by a brass ring with a circular opening of 14 mm in diameter. 0.15 ml heparinized blood is then placed on the upper paper which penetrates also the other five circular papers, that are thus wetted by the blood or the plasma.

There is furthermore an arrangement which permits the insertion of a brass tubule, through which pressures from 5 to 100 mm Hg can be applied. If no pressure is applied, the capillarity of the filter paper may suffice for the determination of RBC filterability. The papers are then each washed in 3.5 ml slightly alkaline water and the hemoglobin concentration of these samples are then determined colorimetrically.

In employing this procedure, Fåhraeus studied the blood of healthy animal and human subjects as well as of patients, afflicted with different diseases, in comparison with the erythrocyte sedimentation rate.

Fåhraeus emphasized the importance of the pore size of the filter paper for his test and chose the Swedish product Munktell Nr. OOH for certain experiments. He also used filter paper of other pore sizes. He then drew the curves for the evaluation of his findings with the six filter papers.

In his paper Fåhraeus criticized strongly Knisely's publication (4) on so-called 'sludged blood'. For instance, Fåhraeus could not accept Knisely's argument that the blood of horses, found to be healthy by Fåhraeus and showing rouleaux of RBC, must have been from diseased horses. At that time I already had observed that RBC can clump either as rouleaux or not in the form of rouleaux. I, therefore, presented in the discussion of the paper by Fåhraeus my concept of intravascular blood cellular clumping or on

the blood sludge of Knisely. Since this discussion of the findings by Fåhraeus, Knisely and my own is, as I believe, of great significance in clinical hemorheology, I am including, as an Appendix to this Editorial, the entire discussion of the paper by Fåhraeus, which contains also the comments on his paper by other participants of this Congress, including the concluding remarks by Fåhraeus.

The Reader may likewise be interested in our later observations both on typical rouleau clumps and so-called 'conglomerate red cell clumps' (5), presented eight years later at the First International Conference on Hemorheology, held at the University of Iceland, Reykjavik from 10. - 16. July 1966.

In the discussion of this paper (5), Roy L. Swank stated that he observed in the living circulation of the hamster's cheek pouch 'irregular very tight aggregation' following large butterfat meals and that RBC rouleaux could likewise be seen. He observed also the 'irregular type of aggregation' in vitro in blood drawn from dogs, rabbits and human subjects that had been given large fatty meals. In addition, RBC rouleaux were observed as well. The non-rouleau type of clumping was also produced by Swank following the injection of high molecular dextran in animals.

As stated in the above mentioned discussion of our paper (5), such irregular non-rouleau clumps of RBC were already observed by me in 1948 after the infusion into patients of a Swedish dextran preparation, named 'Macrose'. Following the infusion, the patients went into circulatory shock which prompted me to abandon this clinical investigation, which I made at the Mount Sinai Hospital in New York City. Fortunately, I could save the life of each of these patients. At that time I was among the first investigators in the United States who conducted the first clinical investigations and animal studies on dextran. Similar non-rouleau clumps of RBC were found by me also in the blood of rabbits infused with this dextran preparation, which, upon further examination in 1948, was found to contain molecules of differing high molecular weights.

In my concept, diagrammatically shown in the Appendix, I referred to blood cellular clumping, because these clumps are not necessarily limited to RBC, but may contain other blood cellular elements as well.

Since in his concluding remarks in the discussion of his paper, Fåhraeus did not respond to the significance of my findings of non-rouleau clumps, I discussed this matter further with him during the Congress at Bad Oeynhausen. He told me that, since he had never observed non-rouleau clumps, it was difficult for him to understand my concept when I presented it. He then expressed the thought that there will be a need to study the formation of such atypical non-rouleau clumping.

More recent studies dealt only with rouleau formation of RBC (6-9), but not with atypical 'conglomerate red cell clumps'.

I consider detailed hemorheological studies of non-rouleau clumping of particular urgency for the understanding and future management of certain circulatory disorders and diseases. It is also for this reason that I consider it important to acquaint the Readers of CLINICAL HEMORHEOLOGY with the entire discussion to the paper by Fåhraeus, presented in the Appendix. Certain points, made in the discussion, are meant to serve as a guide for future studies in clinical hemorheology.

ACKNOWLEDGEMENT

The Author is grateful to Dr. Dietrich Steinkopff Verlag, Darmstadt, F.R.G., the publisher of Rheologica Acta, and its Executive Editor, Professor Dr. H. Giesekus, for kindly granting the permission to reproduce the Discussion of the paper by R. Fåhraeus in toto and in facsimile.

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APPENDIX

Die intravaskuläre Erythrozytenaggregation und ihre Einwirkung auf die Rheologie des Blutes

Von Robin Fåhraeus (Universität Uppsala, Schweden)

Rheologica Acta, Band 1, Nr. 4-6 (1961)

pp. 656-665

Diskussion

pp. 663-665

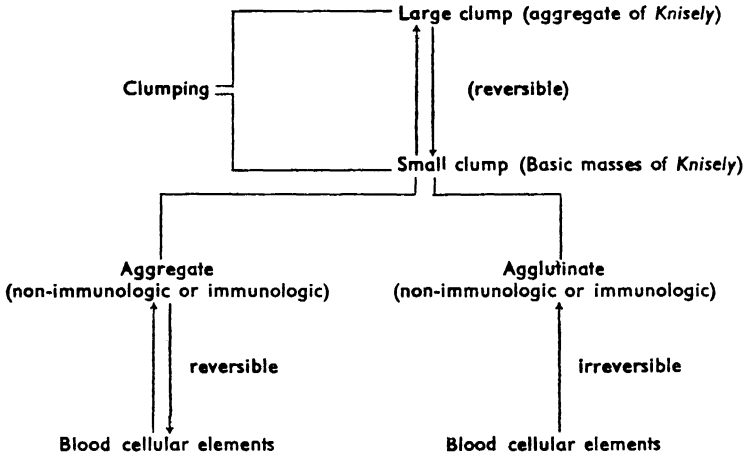
A. L. Copley (London) (with 1 diagram):

I should like to congratulate Dr. *Fåhraeus* on his most interesting contribution. For many years I have been a student of his pioneering work and I find his new approach to problems of the microcirculation by the use of his method of piles of filter papers through the pores of which the blood has to pass, although it might be quite different from blood flowing in capillary tubes as Dr. *Fåhraeus* also mentioned, a stimulating one. I wonder whether he has made studies by the use of varying pressures, high as well as low, for blood to be forced through these layers of filter paper on the one hand and on the other hand by using piles of filter paper of varying pore size and employing the same blood samples for comparative observations. Further, whether there are differences in results by the use of various papers of the same pore size. It seems to me that from such an approach certain new information could be obtained which might be applicable to some of the problems to which Dr. *Fåhraeus* has been referring. When Dr. *Fåhraeus* mentioned blood samples taken from patients with quite different diseases, it is not quite clear to me whether he meant to imply that the particular finding he obtained was pathognomonic or whether the nature of the diseases of these patients was incidental and had no special meaning for the test performed. Further, were these tests on the blood of such patients made in one single case or on many patients with the same or a similar pathologic condition?

There is one particular point which I would like to bring up now, which concerns the work of Dr. *Knisely*, who had hoped to be here but who, unfortunately, could not come. I believe that Dr. *Fåhraeus*' opinion with regard to Dr. *Knisely*'s work, which he mentioned, appears to me to be rather harsh and, I am afraid, not fully justified. I have been studying *Knisely*'s work as well with regard to the so-called "sludge" formation, which, by the way, I do not consider a happy term and which I would rather have replaced by the more general and less committal term of "intravascular clumping". I agree fully with Dr. *Fåhraeus* that such "clumping", as I call it, or as *Knisely* calls it, "sludged blood", cannot be considered without exception as a sign of disease, as *Knisely* claims. However, I can neither accept Dr. *Fåhraeus*' opinion that all such blood clumping is identical with rouleau formation, nor Dr. *Knisely*'s opinion that "sludge" has nothing to do with rouleau formation. My attempt is to bring both views together on this latter question and I strongly believe this can be done. There are some conditions where this intravascular clumping is identical with rouleau formation, that is, an aggregation of red cells, which I consider to be a reversible process, and, on the other hand, there are conditions of clumping which differ from rouleau formation, so-called agglutination, which is, as I see it due to an irreversible process of red cell clumping [*A. L. Copley*, J. Colloid Sci. *7*, 323 (1952)].

This may be shown in a diagram:

Concept of *Copley* on intravascular blood cellular clumping or on the blood sludge of *Knisely*



In the case of the immunologic nature of aggregation and of agglutination the terms would be "immuno-aggregation" and "immuno-agglutination", respectively.

Now, to decide whether we have an aggregation or an agglutination in certain cases I recommend that an arterial blood sample be taken and simply examined under the microscope in order to determine whether or not the red cells are aggregated or reversibly clumped; or, as Dr. *Fåhraeus* would call it, in rouleau formation. On the other hand, it could then be readily shown whether the clumped red cells or sludges are agglutinated or irreversibly clumped as Dr. *Knisely* has all along maintained in his writings.

Last June, when Dr. *Knisely* visited me at my laboratory in London he mentioned, however, that he had lately observed, under certain conditions, that sludged red cells may be seen to be dispersed as single cells in the living circulation. Indeed I was glad to hear him say this, as a great deal of controversy exists in the literature concerning this point. Actually this is a very ancient observation, which *Antony van Leeuwenhoek*[1]) described in a letter in 1699 to the Royal Society in London, concerning studies of the circulation in the capillary bed of the tadpole's tail. Certainly, there are conditions where such clumping or sludged blood is not due to rouleau formation, but to agglutinated red cells, such as e.g. in certain blood transfusion reactions. As I mentioned before I do hope that on this question of Dr. *Knisely* and Dr. *Fåhraeus* can be brought together.

One fact appears to me to be of the utmost importance, namely that both these clumps, aggregates or agglutinates, can block the circulation in the small vessels to such an extent as to induce pathologic changes. Dr. *Fåhraeus* mentioned already in 1921 such a possibility, long before *Knisely* made his interesting observations.

Dr. *Fåhraeus*' studies concern the flow of clumped blood in human subjects through vessels of small diameter and this poses a number of rheological problems, some of which were discussed by Dr. *Scott Blair* in so masterly a way in his presentation on the sigma phenomenon last Friday. The study of the flow of blood presents a number of interesting phenomena. For many years I have been wondering how one single red cell passes through a "true" capillary in the living circulation, where the diameter of such a capillary is smaller than the diameter of the red cell. Surely, this phenomenon must be known to any observer of the living microcirculation. The red cell, which is flexible and shaped like a disc, compressed in the middle, has a diameter of about 7.5 μ and a maximum height of about 2.5 μ. The red cell has no motility of its own, as have certain white cells. In the smallest living capillary, or so-called "true" capillary, which would have a diameter, let us say, of about 5 μ, such a red cell would be markedly deformed while being pushed through the lumen. The pressure alone (probably < 20 mm Hg) in such a capillary, however, would not suffice, it seems to me, to push

[1]) *A. v. Leeuwenhoek* – (spelled Lewenhoek in the original paper) Phil. Trans. Royal Soc. London **22**, 447 (1700).

this cell through the capillary lumen. What, I ask you, could be responsible for the flow of this single red cell? Could it be that the inner lining of the blood capillary might account in some way for its movement? This is a question, which has puzzled me for a long time and which needs a rheological answer. Red cells, whether or not irreversibly clumped, may stick together and can obstruct the circulation in minute blood vessels but we should be aware that a single red cell may hinder the circulation in the smallest blood capillary when stuck in it. The question Dr. *Knisely* and numerous other authors posed in the literature, although it is not of such interest to this audience as to medical people, is whether sludge formation, wherever it occurs, is a sign of disease. I certainly agree with Dr. *Fåhraeus* that this is not necessarily so. On the other hand, I cannot accept the view of *Knisely*'s adversaries, who rule out that there can ever be intravascular clumps leading to a pathologic condition. Here, I should like to emphasize that the occurrence of cellular or plasmatic clots of all kinds, in spite of their obstructing the circulation in blood vessels–such as even one single red cell may do–may not ensue a pathologic condition. Disease will only develop from such obstructions in the circulation if they are numerous enough or if they are located in certain vital organs so that such impairments would develop to such a degree that clinical manifestations or marked pathologic changes become evident.

Finally, Dr. *Fåhraeus*' implication that under certain conditions rouleau formation actually facilitates the flow of blood, appears to be of great practical interest and is a manifestation of the sigma effect. I should like to ask Dr. *Fåhraeus*, in closing my remarks, whether and when he would suggest that means (e.g. intravenous injection of gelatin), in certain clinical conditions in patients, could be used therapeutically to produce such rouleau formation.

In replying to Professor *Reiner*'s question, the red cells, after they pass through a capillary which is narrower than their own largest diameter, do regain fully their original shape, after being markedly deformed in the narrow capillary.

M. Joly (Paris):

Le franchissement des vaisseaux capillaires par les globules rouges est fréquemment accompagné d'une déformation de ceux-ci. Est-ce que des déterminations de la viscosité interne des globules rouges ont été effectuées? Ce serait une donnée importante du point de vue théorique car le degré d'ordre de la distribution des molécules d'hémoglobine à l'intérieur des globules a pu être déterminé par diffusion des rayons X aux petits angles et, par suite, la connaissance de la valeur de la viscosité interne permettrait d'évaluer l'ordre de grandeur de l'énergie d'interaction entre les molécules d'hémoglobine.

R. L. Whitmore (Nottingham):

I wonder whether Professor *Fåhraeus* could give any indication of the type of paper used in his blood filter and whether its porosity or texture was an important factor in the results he obtained?

R. Fåhraeus (Uppsala) Schlußwort:

In conformity with Dr. *Copley* I find "sludge" an unhappy term. The phenomenon to which it refers is namely in my opinion only the consequence of the more or less increased normal tendency of the erythrocytes to aggregate and during pregnancy and diseases brought about by an increase of the plasma globulines. The proof of this conception is the close correlation between the sedimentation rate and the intravascular aggregation of the red cells. I can not see that Dr. *Copley* has stated any new facts which can give rise to an altered explanation. I refer to my paper The Suspension Stability of the Blood (1921).

To Dr. *Copley* and Dr. *Whitmore* I wish to say that the quality of the filter paper self-evidently is of great importance in experiments of this kind.

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