THE ELECTROPHORESIS OF THE BLOOD PLATELETS OF THE HORSE WITH REFERENCE TO THEIR ORIGIN AND TO THROMBUS FORMATION.

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Little is known of the physicochemical characteristics of blood platelets. There has been much dispute concerning their origin. The study of their cataphoresis affords a means of determining a fairly definite physicochemical constant which defines the make-up of these bodies in specific channels. Such data on the cataphoresis of the blood platelets of the horse and some other incidental observations, form the basis of this communication.

Review of the Literature Concerning Origin of Platelets.

The following review merely indicates the variance of opinion. It is incomplete.

In 1906 Wright (1) maintained that the cytoplasm of megacaryocytes was parent to that of blood platelets. Bunting (2) and Ogata (3) confirmed Wright's work. Brown (1913) (4), however, showed that hyperplastic endothelial cells in the marrow, and mononuclear and transitional cells in the marrow, spleen and blood, could also give rise to blood platelets.

Menne (5) made a specific immune serum from leucocytes and platelets and concluded from his studies that the structures of the leucocyte and platelet vary. Perroncito (6) (1920) believed that platelets arise from the red and white cells of the circulating blood. Schilling (7) (1921) came to the conclusion that the nuclei of red cells play a significant rôle in platelet formation. Erede (8) (1921) rejected the megacaryocyte origin. Rosenthal and Falkenheim (9) (1922) performed rather careful and well controlled experiments. They found that an erythrocytic immune serum was highly agglutinative for red cells but had a comparatively

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negligible influence on platelets. Platelet antisera also failed to agglutinate red cells. They concluded that, from the point of view of receptor structure, platelets and red cells present significant differences, while with the cells of the leucopoietic system the platelets possess a common receptor system indicating a common origin for the platelets. Marchesini (10) (1923) maintained that platelets arise from degenerated red blood cells which are phagocyted by megacaryocytes and then transformed by these cells into the form found in the blood. A rather complex hypothesis has been offered by Demel (11). This theory seems to hold for the origin of these cells (platelets) by a precipitation process direct from the blood plasma. The process is to be governed by megacaryocytes in the presence of physiological necessity. Further evidence in support of Wright's view was given by Katsunuma (12) (1925). Petri (13) in the same year denied the validity of Wright's views. Stahl, Horstman and Hilsnitz (14) by means of an iodine fixation method showed that certain granules were specific for platelets and megacaryocytes, again supporting Wright.

Method.

A modification of the Northrop (15) cataphoresis cell described elsewhere by Freundlich and Abramson (16) was used.

The plasma was oxalated by the addition of 8.5 cc. of a saturated solution of K oxalate per liter of horse blood. The platelets remain well preserved in the ice box for at least 48 hours.

The velocity of the platelets and of the polymorphonuclear leucocytes was determined as follows: The study of red cell migration at different levels in the cell permits the estimation of V_w, the velocity of the water in the midregions. The velocity of another particle in the midregions is then expressed by the equation

$$V_o - V_w = V \tag{1}$$

where V_o is the observed velocity of the particles and V is the absolute velocity. The reader is referred to previous communications for further data on method and related phenomena of cataphoresis (16, 19).

The Migration of Single Platelets.

The data of Table I are from six different horses. The mean velocity for the platelets is .45 μ per sec. per volt per cm. Polymorphonuclear leucocytes migrate with the same speed, within the limits of experimental error. In fact, simple observation confirms the measurements. Practically no difference in speed is observed. It may be recalled that in plasma lymphocytes migrate 15 to 30 per cent, and red cells about 90 per cent faster than leucocytes (19). The

same relationship holds therefore for blood platelets. Considering the difficulties of the method, the high conductivity of the medium and the low electrokinetic potential, with the exception of Plasma 2, the agreement in the five other specimens is excellent. It is surprising that blood platelets which are supposed to be so fragile retain for so long the same surface characteristics as far as the electrokinetic po-

TABLE I.

The Cataphoresis of Platelets in Plasma.

The speed of polymorphonuclear leucocytes is given in the last column. Although red cells and small lymphocytes have different velocities, note that platelets and polymorphonuclear leucocytes have the same velocity (six horses).

Plasma	Age	Platelets		Polymorphonuclear leucocytes
		V_o	V	V
	hrs.	*μ per sec. per volt per cm.*	*μ per sec. per volt per cm.*	*μ per sec. per volt per cm.*
1	30	.71	.41	.46
2	6	.82	.59	.57
	30	.76	.55	.60
3	6	.65	.46	.52
4	6	.57	.40	.43
5	6	.67	.51	.53
6	6	.68	.46	.54
	Mean excluding No. 2		.45	.49
	ζ potential (millivolts) (26.5 × μ per sec. per volt per cm.)		12	13

tential is determined by this surface. One is almost led to believe that their surface is determined by the presence of the plasma proteins rather than by an inherent composition.

The Migration of Aggregates of Platelets.

According to classical conceptions (17) the ζ potential of agglutinated blood platelets should be lower than that of single cells. In

specimens 30 hours old clumps of from 5 to about 20 platelets have been studied. There is no appreciable difference between the cataphoretic velocity of these aggregates and that of single cells. It is possible that some change in the ζ potential takes place incidental to the withdrawal of the blood. Just how far the aggregates observed represent a mechanism of slow coagulation by the particles below the critical potential must be reserved for future discussion.[1] The formation of aggregates without change in electrokinetic potential has been reported previously by Freundlich and Abramson for red cells (16).

Another outstanding feature of platelets is that they are able to stick to the glass walls of the cataphoresis cell with a remarkable tenacity. (That platelets are "sticky" has, of course, been noted hitherto.) This force is so great that a stream of water sucked through the cell does not remove them. The same adhesive quality has been discussed previously for leucocytes (19, 20). On the other hand, it is curious that neither red cells nor lymphocytes under the same conditions are possessed of similar properties. This fits in remarkably well with the behavior of all four types of cells in the presence of injury to tissue or capillary wall.[2]

DISCUSSION.

It would seem from the preceding data that the surface of polymorphonuclear leucocytes and that of blood platelets are similar. The electrokinetic potential is the same for both in a highly complex medium. This is all the more striking because of the fact that lymphocytes and red cells have a cataphoretic velocity which is unmistakably greater. Offhand one is inclined to believe that the platelets and leucocytes have a common leucopoietic origin. This would fit in quite nicely with the theory of Wright as follows: The blood platelets arise from the megacaryocytes which have in their

[1] Polymorphonuclear leucocytes and even quartz particles form aggregates whose cataphoretic velocity is the same as that of single particles with the same suspension. This "isopotential" agglutination will be discussed further in a future communication.

[2] This point is discussed in detail in an article by the author to be published (Abramson, H. A., in Alexander, J., Colloid chemistry, New York, ii).

turn been derived from myeloblastic cells (21); and the transition from myeloblast to leucocyte is, as far as concerns surface change, probably not a complex one. Still, one should accept this rather convincing evidence with a certain amount of hesitancy as it has been found in further experiments that quartz particles migrate with the same velocity in serum as leucocytes.[3] It has been also found that such quartz particles are influenced by slight traces of proteins (*e.g.* 10^{-7} gelatin solution lowers the ζ potential of quartz appreciably (18, 22)). Now, whether the white cells and blood platelets act like an inert particle, surrounding their naked protoplasm or cell membrane with a sheath of the protein in the medium, or whether they have acquired during their development the surface giving them their charge, is a question which is intimately bound with studies of surface adsorption and cataphoresis of these blood units. Experiments on the point have been started. At any rate, one can say that platelets and leucocytes have similar surfaces—certainly slightly different from lymphocytes and very different from red cells. And one may assume with a fairly reasonable degree of certainty that the unchanged relationship through the development of both types is strongly suggestive of a common origin.

The stickiness of the blood platelets has been noted. It must be remarked in concluding that the so called glass surface of the cataphoresis cell is really covered by a more or less complete layer of protein in the presence of even small protein concentrations (18). The platelets are really adherent to a protein film. The magnitude of this adhesive force has in general been described. It has been mentioned that aggregates are formed without changing the ζ potential. With this in mind, one may look upon thrombosis from the following point of view. Incidental to injury of the vessel wall, the adhesive force possessed by platelets is sufficient to permit them to remain attached to the wall in spite of the flow of blood rushing by. No change in electrokinetic potential is needed to establish a state of aggregation. Aggregation can probably occur in plasma without any measurable change in the ζ potential. The piling up of blood platelets can easily be explained

[3] The data and connected theory will be given in Abramson, H. A., *J. Gen. Physiol.*, 1928, xi, in press.

by the stickiness of the cells themselves, produced by adsorbed or inherent protein films. The same mechanical, simple conception may be applied to the subsequent addition of leucocytes to the thrombus and the attachment of fibrin strands.

SUMMARY AND CONCLUSIONS.

1. The cataphoretic velocity of blood platelets (horse) in plasma has been found to be between .40 and .51 μ per sec. per volt per cm. The mean velocity obtained from five horses is .45 μ per sec. per volt per cm.

2. The cataphoretic velocity of polymorphonuclear leucocytes in similar specimens is practically identical with that of the platelets. This is noteworthy because of the fact that lymphocytes and red cells have different speeds.

3. With spontaneous agglutination of platelets, white cells and red cells, there is no change in the cataphoretic velocity incidental to aggregation.

4. The possible surface composition of platelets and white cells is briefly discussed.

5. The bearing of these findings on the origin of blood platelets and the mechanism of thrombus formation is demonstrated.

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