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New observations on the role of the erythrocyte in hæmostasis

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SYNOPSIS

Classical expositions of the theory of blood coagulation hold that consolidation of the fibrinous reticulum is due to the mechanical enmeshment of erythrocytes within it. Studies of coagulating blood by dark-ground microscopy have revealed the attachment of submicroscopic threads to the erythrocyte walls at precise loci: it is postulated that the formation of captive cells (*penderocytes*) leads to hæmostatic occlusion and the total retention of erythrocytes within the contracting coagulum. Filmed records of the phenomena provide distinction between true penderocytes, those erythrocytes attached at random, and occasional enmeshed cells.

The erythrocyte has been held to take no active role in the phenomena of hæmostasis since the view revived as recently as 1932 by Watson that platelets arose from them. Microscopical examination of the mechanical processes involved has been carried out in the past by such means as hanging-drop preparations described by Harris (1934) or by sealed preparations such as those described by Brecher and Cronkite (1950). Hæmostasis is essentially a dynamic phenomenon: it is only by the examination of flowing blood that hæmostasis can be successfully studied, yet blood flow is precluded by these orthodox techniques.

METHODS

Human blood from subjects with a "normal" blood picture was diluted with twice its volume of physiological saline in a Pasteur pipette and mounted between a fine cover-glass and a 3″ × 1″ slide previously cleaned in Na_2CO_3 soln, boiled, and dried in ethanol. The preparations were examined by means of dark-ground microscopy with an oil-immersion objective NA 1·30 stopped to NA 1·00 using × 8 eyepieces for ciné micrography and × 10 and × 15 eyepieces for visual observation and still photographs. Illumination was by means of a 300-watt projector unit forcibly cooled; this produced a sufficiently intense source of light for dark-ground ciné micrography and at the same time emitted little infra-red radiation. Results were recorded on 35 mm HP3 film for still photomicrographs at exposures of 1/50th sec to 1/5th sec, and cinémicrographs were obtained on 16 mm FP3 in a modified G45 camera filming between 2 and 25 fps.

OBSERVATIONS

The observations concerned only the processes of actual arrest of blood flow, induced by cautious pressure on the periphery of the preparation cover-slip. The

detailed study (Ford, 1965) commenced after fibrin threads were visible in the specimen.

At this stage (fig. 1) the denser areas of the preparation are largely occluded by the fibrin coagulum. Pressure on the cover-slip allows flow to carry the free erythrocytes through the threads with little impedence. In a contracting meshwork this mechanism would lead to the liberation of the majority of erythrocytes into the expressed serum: the retention of the overwhelming majority within the meshwork which occurs must therefore be due to another mechanism. Evidence of such a mechanism, hitherto unsuspected, was obtained from observations initially carried out in the least dense areas of the preparation: erythrocytes were observed to be retained in the manner of a balloon on a string apparently by a single thread of submicroscopic proportions. In view of the dimensions of the thread it was not initially directly observable visually but its presence could be inferred. The criteria employed to distinguish erythrocytes held captive in this manner are listed below: they serve to

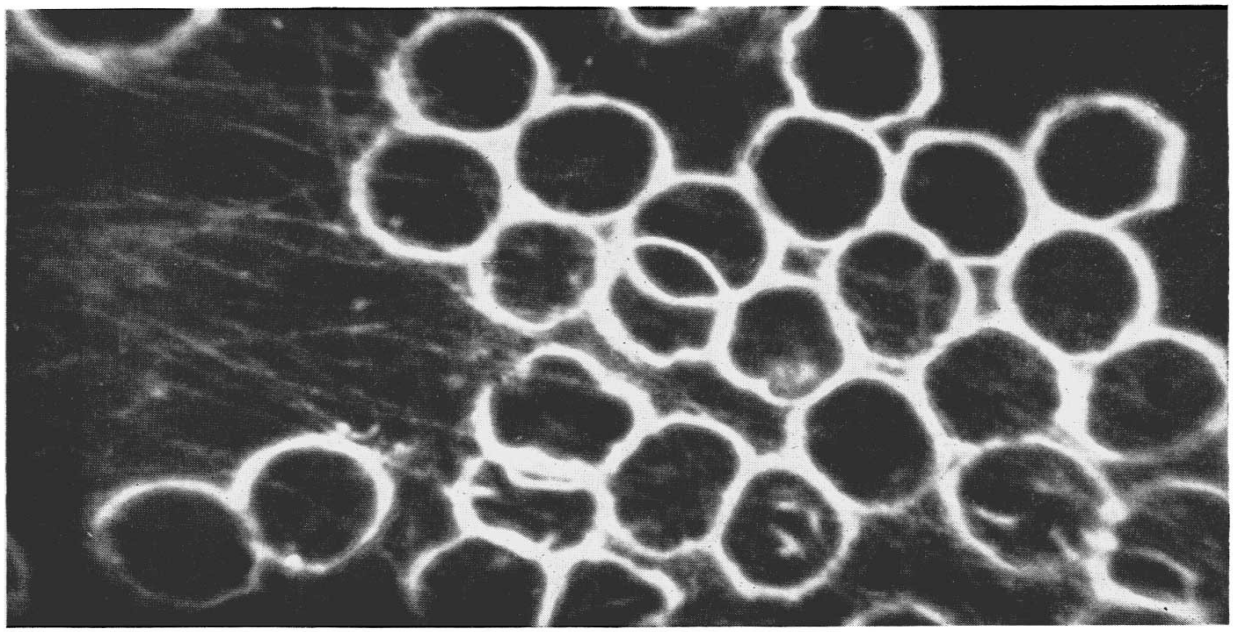

Fig. 1. Erythrocytic distortion during flow through the fibrin reticulum. × 2,000.

differentiate the true captive cells (referred to as "*penderocytes*") from cells attached to the slide surface or otherwise held by random attachments.

1. *Flow-dependent reversibility*

This phenomenon (fig. 2A) is elucidated when "pear-shaped" penderocytes are observed to be held stationary against a background of moving cells. They are seen to be orientated with the apex directed against the direction of flow. When flow ceases the cell regains its circular appearance; reversal of the direction of flow produces a similar condition but orientated at 180° to the original state. The thread (drawn in the diagrams fig. 2A, B & C) though not directly observable, is clearly demonstrated since this technique alone gives unequivocal substantiation of its existence.

2. *Flow-dependent thread elasticity*

Elastic changes of penderocyte configuration and of the length of the sub-microscopic thread are produced by varying the rates of flow. The apical point of the penderocyte becomes increasingly acute as rates of flow increase, a change which is completely reversible. (See fig. 2B).

3. *Lateral displacement phenomena*

As the penderocyte is "jostled" by flowing unattached cells, it describes an arc in its lateral movement, subtended by the sub-microscopic thread which holds it captive. (See fig. 2C).

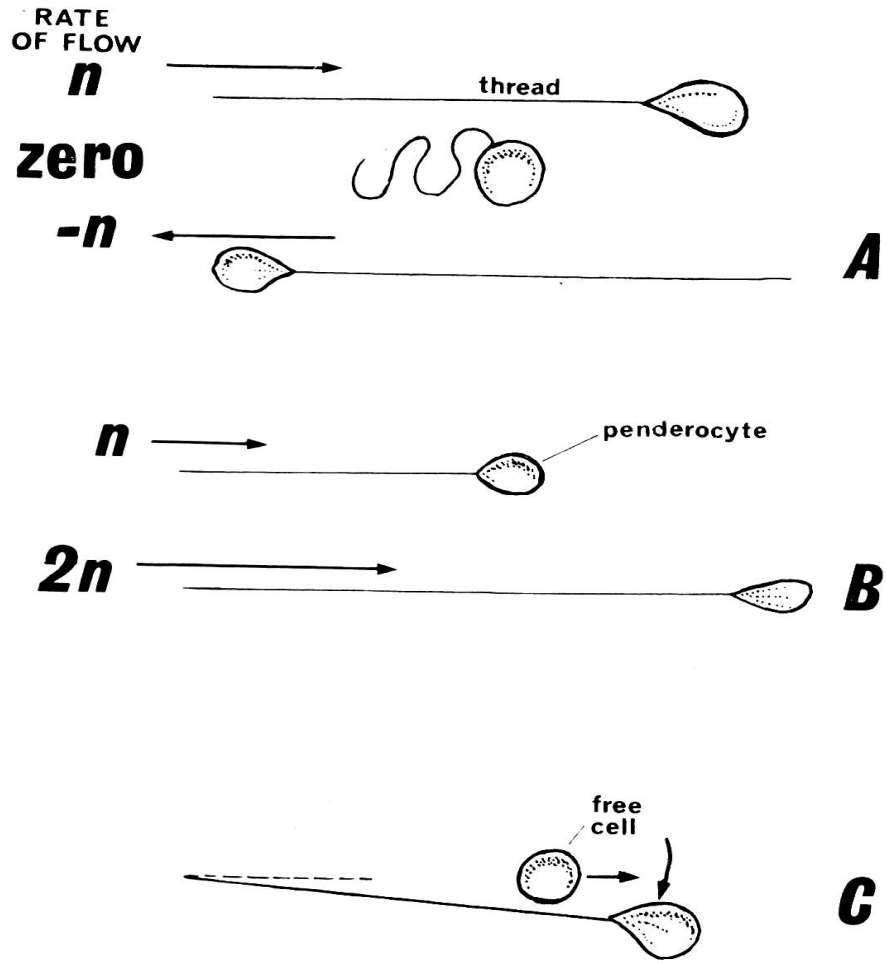

Fig. 2. Diagrammatic representation of erythrocyte capture (for explanation see text).

Groups of penderocytes (shown in fig. 3) increase in number as time goes by until the majority of erythrocytes are captive in this way. Large groups of them may be observed and have been filmed on 16 mm ciné film (Ford 1963). It is suggested that by means of this phenomenon of penderocytic occlusion hæmostasis occurs and eventual retention of the erythrocytes within the contracting coagulum is effected. Were the mechanical enmeshment of the erythrocytes alone responsible for their retention, then (in view of their demonstrable flexibility and their manifest ability to pass through gaps barely 2 μ across) a far higher percentage would be visible in the free serum expressed from the coagulating mass.

Under conditions of very high rates of flow, the submicroscopic thread may part under the strain. As the erythrocyte drifts away its wall may be seen to oscillate in and out due to the elastic forces released by the precipitate breaking of the thread. Such cells may be studied when they are at rest in a dilute region of the preparation. The thread, of increased diameter due both to the further precipitation of fibrin

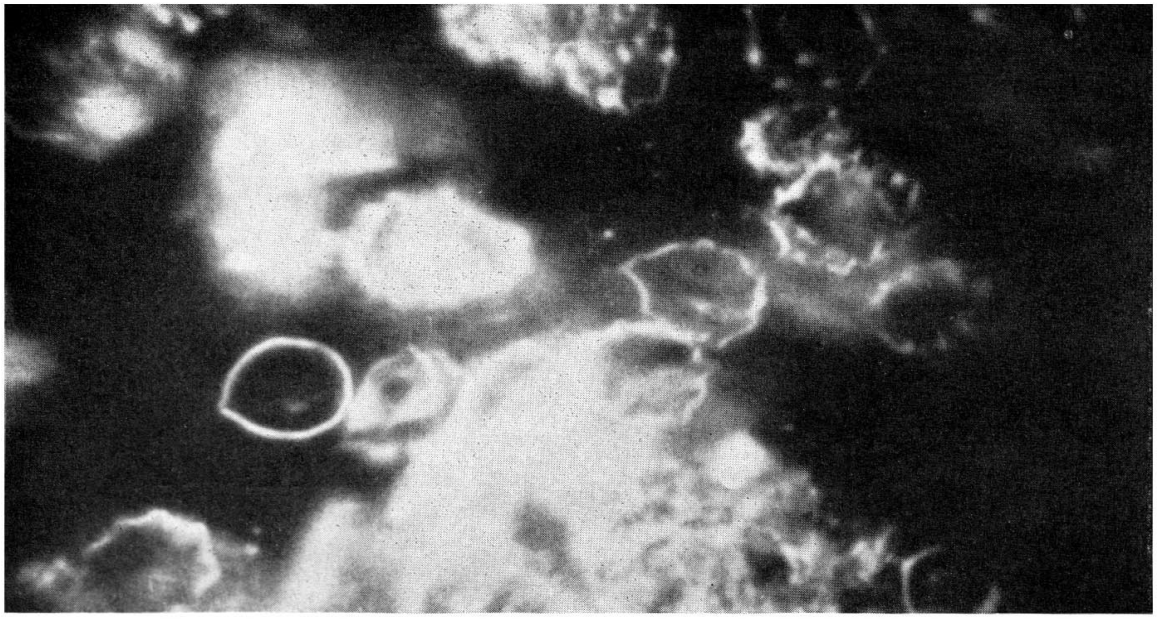

Fig. 3.—Penderocytes stationary against direction of induced flow (moving erythrocytes show as blurred images).

and the release of longitudinal tension forces, may be resolved by critical dark-ground microscopy as an undulating fibril in a pronounced state of Brownian agitation. Several examples of these have been filmed.

There are many anomalous phenomena associated with hæmostasis and its irregularities (Biggs & MacFarlane, 1962) which centre around the failure of blood to initiate coagulation successfully under several conditions that are not fully understood. It is postulated that the fixation of erythrocytes in this way and the phenomena of penderocytic occlusion that result may help to assist the interpretation of some of those anomalies which are still unresolved.

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