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Rheological Factors in Platelet-Vessel Wall Interactions [and Discussion]

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Rheological factors in platelet – vessel wall interactions

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Rheological aspects of platelet–vessel wall interactions involve cell–cell encounters, platelet – vessel wall encounters and platelet–thrombus interactions. The cell–cell encounters are usually caused by convection of cells in shear flows rather than by Brownian motion; this is important in aggregation and in the enhancement of the diffusion of platelets by red cell motion. Platelet – vessel wall interactions can involve transient adhesion (lasting from a fraction of a second to a few minutes) as well as more permanent adhesion. Reaction rates between platelets and walls are generally very small except on damaged vessels and some artificial surfaces. Ultrafiltration through the vessel wall affects cell–wall interactions. Rheological analyses of thrombus formation have been made and show interesting relations to experimental observations. Some experimental results have indicated that platelets are capable of reacting within a small fraction of a second. Red cells may act as mechanoreceptors for increases in shear rate and facilitate the speed of response of platelets. Surface geometrical forms such as bumps and cavities tend to prolong residence times and facilitate thrombus formation.

1. Introduction

The interaction of platelets with the vessel walls is a dynamic process. The motion of the bloodstream and rheological factors must be considered. Indeed, according to some hypotheses concerning the processes leading to haemostasis, certain rheological events are essential factors. Like the biochemical processes now increasingly understood, rheological processes are invoked as necessary components in accounting for haemostasis and thrombosis.

This acknowledgement of rheological factors, the fluid dynamic events, is a departure from Virchow's triad. It is an amplification rather than a contradiction, posing new questions and the development of new techniques in the study of platelet – vessel wall interactions. The recognition of rheological factors is by no means new: Mustard *et al.* (1962), is an extensive review of thrombus formation *in vivo*, point out that the opinion that mechanical factors have a role can be traced back into the late nineteenth century (Welch 1887).

A major stimulus for study of platelet–wall interactions has been the observation that many myocardial infarctions follow the development of major thrombus tied to an atheromatous plaque in a coronary artery, that related processes in carotid vessels lead to strokes, and that thrombi develop at vessel–prosthesis anastomoses and at junctions in extracorporeal blood circuits. Indeed, the development and use of artificial heart valves, artificial kidneys and prosthetic blood vessels since about 1960 has coincided with a period of intense study of platelet behaviour. It has also coincided with a renewed interest in blood rheology, and it has become increasingly clear that flow phenomena on the scale of the cells in blood have needed more attention than had been previously given. The name 'microrheology' has been given to the study of flow at the scale of the blood cells, and the name 'macrorheology' to the study of bulk flows in which the blood is considered to have simple averaged properties. Both represen-

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tations are important in the understanding of rheological effects in platelet–wall interactions. The period of intense study of platelet behaviour has also coincided with the introduction of several new and refined techniques such as scanning electron microscopy and high-pressure liquid chromatography.

Studies of platelet biochemistry, of thrombus micropathology, of blood rheology, of artificial organs and of clinical control of risk factors for myocardial infarction and stroke have coincided in time but they have not been closely coordinated. In providing an account of the role of rheology, the development of some significant stages in thrombosis research will be examined in relation to studies of fluid dynamics and convective transport.

2. Development of the feedback hypothesis

Mustard *et al.* (1962) pointed to injury to the vessel wall as a mechanical factor predisposing to thrombus formation, and suggested that atheromatous lesions may be consequences of antecedent mural thrombi; further, they point to the effects of flow distribution such as bifurcations and small ridges in producing thrombotic deposits. Most significantly, in discussing the growth of a thrombus, they infer that the rate of growth of a thrombus should in some way be proportional to the size of the thrombus, and that it might not be surprising to find something like an exponential growth.

At the same time, the technique of aggregometry was introduced (Born 1962), and this method for observing platelet response to applied factors by measuring light transmission through a small tube of platelet-rich plasma was found to be a powerful tool for research *in vitro*. The small cuvette has a stirrer creating a rotation – a shear flow field – which raises the inter-platelet collision frequency well above that which would occur by Brownian motion. The flow field is not uniform and has a range of shear rates rather than a single value. Because of this it is not practicable to use the instrument to measure collision efficiency (the fraction of encounters between platelets that result in aggregation of the encountering cells), as this is expected to vary with the shear rate, but the instrument has shown itself to be a fine device for the study of factors that promote aggregation and those that inhibit it. The average time interval between platelet collisions is of the order of 3 s, and this allows the device to respond quickly and to complete a test in a few minutes. It has been shown subsequently that factors that promote platelet aggregation in platelet-rich plasma (p.r.p.) also promote it in whole blood, and that inhibitors of aggregation in p.r.p. act similarly in whole blood. Indeed, it has been demonstrated (Richardson *et al.* 1976) that regional application of drugs that inhibit aggregation in p.r.p. can be used to reduce thrombus formation in artificial organs through which whole blood passes. In this sense, the necessary absence of the red cells from the aggregometer is not misleading in assaying the effects of chemical factors on platelet aggregation. However, it has been found that thrombus growth rates are affected by the presence of red cells, an effect attributed at first to enhancement of platelet diffusivity by red cell motion – a purely rheological effect – but now considered to involve the red cells biochemically as well. This is discussed further later.

Two aggregometer systems were developed subsequently to make aggregation measurements under conditions of well-defined flow. Chang & Robertson (1976) used a rotating concentric cylinder system, and were able to deduce collision efficiencies. Rieger (1976), working in the laboratory of Schmid-Schönbein in Aachen, used a rotating cone-and-plate system (sweeping

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through shear rates of 4.5–115 s^{-1} in 12 min) and obtained data on the effect of shear rate in a continuous rather than discrete manner. Both systems have relatively large surface:volume proportions.

Animal models provided opportunities for observation of thrombus growth. In the microcirculation (e.g. hamster cheek pouch, mouse mesentery, rat mesentery, rat cremaster and bat wing) it was possible to record thrombus growth on an injured vessel site by using a 1 mA

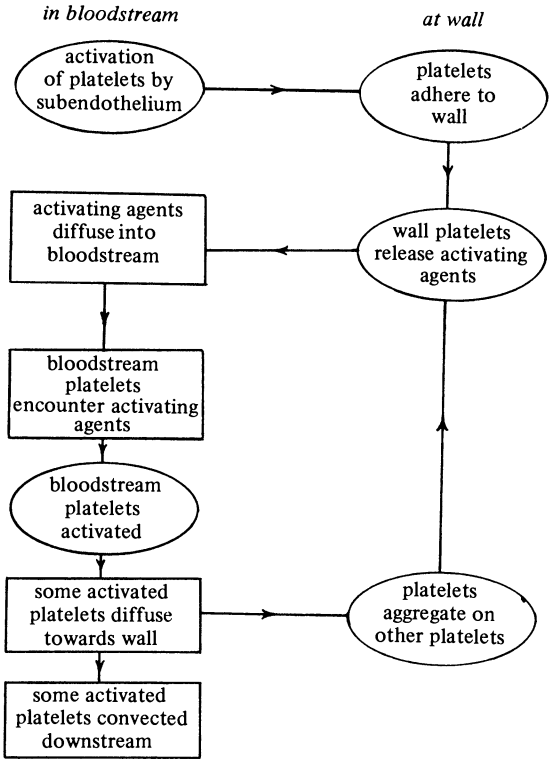

FIGURE 1. Hypothesis (*ca.* 1970) concerning the interaction between the bloodstream and the vessel wall, leading to thrombus formation. Events in the bloodstream are shown in the left column; events on the wall are shown on the right. Rectangles enclose events that are predominantly physical in nature, while ovals enclose those predominantly biochemical.

discharge or a laser to induce vessel damage. A model involving a larger vessel was provided by the rabbit aorta de-endothelialized by balloon; basement membrane was exposed and platelet-dominated thrombi grew on the denuded vessel wall (Baumgartner 1973).

Numerous studies demonstrated the importance of calcium and of fibrinogen as essential factors in platelet adhesion and aggregation; platelet adhesion to collagen was also shown. The aggregometer and electron microscope showed how platelets are activated by an adequate concentration of adenosine diphosphate, with morphological changes leading quite rapidly to the development of pseudopodia. After the work of Bangham & Pethica (1960) it was perceived that the pseudopodia would facilitate adhesion of the cell membrane, e.g. by reducing the effect of Coulomb repulsion forces. It was also realized that the deployment of pseudopodia would increase the effective cell radius and thereby increase the collision frequency in a shear flow. Later after platelet activation there is the release reaction (specifically inhibited by ASA)

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in which platelet granules discharge into the plasma factors that themselves activate platelets (ADP serotonin).

These observations were put together to support a thrombosis hypothesis incorporating feedback. It is illustrated in figure 1. This hypothesis was a natural sequel to the suggestion of Mustard *et al* (1962). The figure has been drawn to give distinguish between processes that are predominantly physical or predominantly biochemical. Some of the physical processes are controlled by blood flow.

3. Thrombus growth rate and the activation delay time

A significant step was the experimental measurement of thrombus growth rates. An animal model was developed by Begent & Born (1970), who showed that it was possible to stimulate thrombus growth in venules by iontophoretic application of ADP in the tissue space just outside the vessel. The thrombus attached to the vessel wall and grew by capture of passing platelets. The model was reversible; with cessation of the stimulus the thrombus embolized and no further thrombus formed at the site unless ADP was reapplied. Morphologically, the vessel wall appeared to preserve an intact endothelium.

Begent & Born applied intravital morphometry to observe the growth rates of the thrombi that they induced. Each thrombus, developing in the vessels of about 50 μm internal diameter, was found to increase in volume exponentially with time. This showed that the speculation of Mustard *et al.* (1962) was well founded. Begent & Born went further by observing the effect of different blood flow rates on the exponential growth. They found that the time constant of the exponential growth varied with the blood flow rate. At low blood flow rates, the growth rate factor increased with blood flow rates until it reached a maximum. At higher blood flow rates the growth rate factor decreased, and at the extreme it went to zero. Richardson (1973) analysed this problem in terms of the theory of aggregation in shear flow (due to Smoluchovski and described by Levich (1962)) and interpreted the low flow-rate results as indicating that the thrombus was enlarged by capture of all platelets that approached it closely enough as they were swept along in the blood flow. To explain the higher flow rate results, Richardson proposed that platelets have a finite time delay between activation and development of a sufficient adhesive force to allow successful aggregation. It was assumed that for each platelet the clock is started on the first moment of encounter with the thrombus. If platelets reached the end of the thrombus in a time less than the activation delay time, they would not adhere to it (although they might adhere somewhere downstream). With a shear flow in the vessel, the platelets approaching the wall most closely generally experience the longest contact times. The activation delay time was deduced to be 0.1–0.2 s from the hamster cheek-pouch experiments.

More recently, Predecki *et al.* (1980*a*, *b*) have used an entirely different technique *in vitro* to assess the activation delay time of human platelets and have deduced it to be 0.3–0.5 s. Their technique used pulsating flows of physiological solution through porous plates supported in whole blood; by controlling the frequency of pulsations they could limit the duration of contact between blood cells and each porous plate, and examination of the plates for adherent platelets allowed the activation time to be assessed.

These estimates for activation delay time (which may vary between species, and also with drugs and so on) are on a timescale that is not readily measurable in cell–cell encounters in aggregometers because of the relatively long time between collisions and also the finite time

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required for the injection of an activating agent (such as ADP) into the cuvette and mixing with the p.r.p.

Born & Richardson (1980), in examining the activation delay time further, pointed out that the theory indicates that the thrombi formed at the higher flow rates would be less thick (for specific length in the direction of flow) than those formed at lower flow rates, and found that this was demonstrated by the observations.

4. Thrombus growth and red cell involvement

The thrombi whose growth rates have been measured in microcirculatory experiments have been quite small, about 50–100 μm in length. The mean spacing between platelets in blood is of the order of 15 μm, so that the thrombi do not differ much in size from the mean spacing between platelets, especially in the early stages of thrombus growth. If one compares the frequency of platelet–platelet encounters by convection in a shear flow with the frequency of encounter by Brownian motion, the ratio of these frequencies (the Smoluchovski number) is of the order of 10^4 for the experiments of Begent & Born (1970). These numbers indicate that, for the small thrombi, an analysis that assumes the convective transport of platelets is dominant and one that ignores diffusion of platelets (associated with their Brownian motion) is reasonable. However, if thrombi can grow much larger, 1–10 mm or so, then the depletion of platelets from the blood passing immediately over a thrombus will be compensated for by a net diffusive flux of platelets towards the regions where the free platelet concentration is low, and thrombus growth downstream can be dominated by the diffusive flux. Thrombi of this size can be formed in flow chambers built for the purpose (as well as in coronary arteries). Experimental data on thrombus growth rates in such chambers have indicated that growth could occur faster than would be expected from ordinary diffusive transport of platelets, and it was inferred that the red cells in blood were serving somehow to enhance the diffusivity of platelets, and indeed by a factor of 10^2 or so. This rheological process has been confirmed in several ways.

The augmentation of diffusivity by red cell motion has been demonstrated by observing the motion of specific cells as they move through a tube (Goldsmith & Karino 1966) and by carrying out a Taylor interface diffusion experiment with flow in a tube (Turitto *et al.* 1972). The self-diffusion coefficients of spherical and disc-like particles in a shear flow of suspensions of particles of various volume fractions have been measured by Eckstein *et al.* (1977) with the use of a concentric cylinder cuvette apparatus. Fischer & Richardson (1980) have illustrated how small tracer particles introduced in a steady shear flow around an isolated red cell experience lateral movements (in the direction across the shear flow) when they come close to the red cell. These lateral displacements provide a mechanism for augmenting diffusion. The particle motions are similar in form to the streamlines described by Poe & Acrivos (1975) for flow around a sphere and a cylinder in a shear flow. As the volume fraction of the suspension is increased, the interval between lateral displacements induced for each particle should be reduced, so that the effective diffusivity is increased. At the large particle volume fractions in blood (i.e. at normal haematocrits) it is not clear how much mutual interference arises to limit the degree of enhancement of diffusion. The experiments of Eckstein *et al.* and of Goldsmith & Karino indicate there is a limit at high particle concentrations. In any case, the rheological phenomenon of enhancement has been demonstrated clearly.

The fact that there is platelet diffusion enhancement in a shear flow does not mean that thrombus growth is equally enhanced. Very small thrombi do not benefit from it. The process

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of attachment of a platelet to a surface bounding the fluid flow is controlled partly by the reaction rate between platelets and the surface. It is controlled partly by the diffusion process, which brings platelets close to the surface and which determines the concentration available in the blood in contact with the surface. In some circumstances the principal limitation in attachment is the reaction rate between the platelets and the surface, and whether or not the platelet diffusivity is enhanced is then of no account. Robertson & Chang (1974) took this approach in making an analysis of the behaviour of blood passing through a bead column, for which platelet retention is affected strongly by the presence of red cells.

There are many pieces of evidence pointing to a chemical involvement of red cells in thrombus formation and platelet adhesion. Some of the evidence suggests that there is a release of intracellular ADP, which activates the platelets. Born *et al.* (1976) investigated the bleeding time at cuts made in thin plastic tubing through which anticoagulated blood was flowing. The bleeding time was very long when p.r.p. was used and red cells were absent. It was much shorter when the haematocrit was normal. It was prolonged when apyrase (which catalyses the dephosphorylation of ADP to AMP) was present. It was prolonged when chlorpromazine had been added to the blood: it is known that chlorpromazine at the concentrations used increases the mechanical resistance of red cells to hypotonic haemolysis while it does not affect platelet aggregation. (In some recent experiments on adhesion of human platelets, it was found also that chlorpromazine did not alter adhesion rates.) Born (1977) has summarized other experiments that indirectly indicate an involvement of red cells in platelet adhesion via ADP.

This has led to the development of a newer hypothesis for thrombosis at cut vessels, which invokes an essential rheological factor: under conditions where blood leaks through a cut, there is a sudden increase in shear close to and in the cut, which can cause enough change in the red cells that they release, *inter alia*, ADP. This hypothesis in illustrated in figure 2, and the next section takes up the testing of the hypothesis in detail.

5. Cells under shear: chemical consequences

The experiments of Born *et al.* (1976) provide a critical challenge to previous hypotheses about thrombus formation. The most critical aspect of the observations lies in the following facts. When blood is pumped steadily through the plastic tube, and the tube is free of cuts or holes, the blood flows steadily through and exits from the end without forming a haemostatic plug. For that matter, the effluent blood is free of platelet aggregates. When a cut or hole is made in the side of the tube, there is a disturbance in the flow so that after a period of blood loss, a haemostatic plug forms and seals the hole; in the blood that flows on through the tube to its end there are some platelet aggregates that have probably embolized from the lumen of the tube in the vicinity of the site of the cut. With the *in vitro* system used, there is no possibility of invoking effects of collagen exposed by the cut, or of the absence of protective endothelial cells, or even the possibility of communication to proximal blood flow via extravascular tissue. The flow is steady rather than pulsatile so that there is no possibility of upstream convection of activating factors as might occur during a pulsatile motion. It would therefore seem that the flowing blood reacts to the stimulus of the cut through mechanical effects created in the vicinity of the cut, and that red cells are strongly involved in that reaction.

The nature of the reaction is the subject of hypothesis and testing. One hypothesis is that the red cells are subject locally to haemolysis, and release intracellular adenine nucleotides with

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ADP in sufficient proportion to activate the platelets: it is a convenient feature of steady tube flows that the highest shear stresses are closest to the wall. Another hypothesis is that the red cells may be deformed sub-haemolytically yet sufficiently to release adenine nucleotides through pores opened in the red cell membrane by the deformations associated with increased shear. Yet another hypothesis is that the shear stress in rising transiently causes a transient potentiation

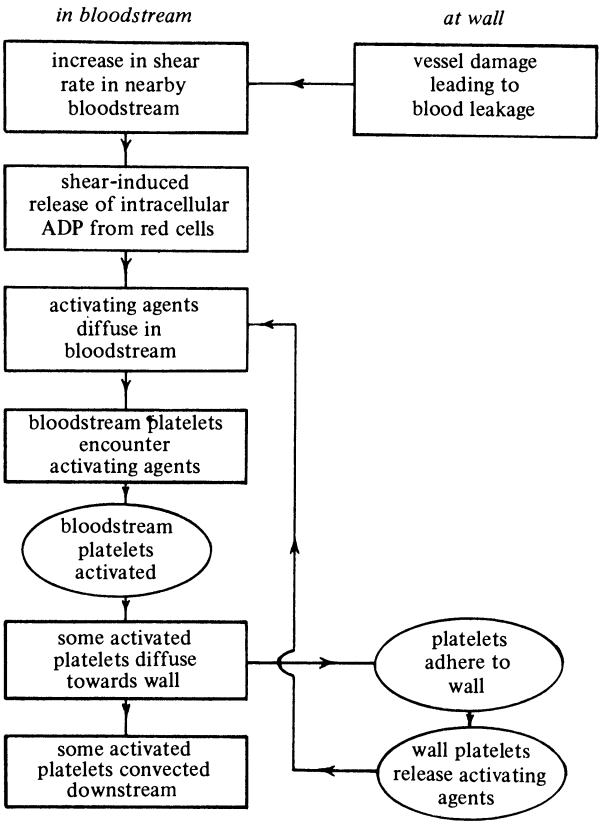

FIGURE 2. Hypothesis (*ca.* 1975) concerning the interaction between the bloodstream and the vessel wall, leading to thrombus formation, especially after arterial puncture. Symbols as in figure 1.

of platelets for adhesion and aggregation presumably by membrane events, e.g. the transfer of a potentiator from the red cell to the platelet when the red cell is stressed enough. These hypotheses are not mutually exclusive.

Two approaches have been taken to testing these hypotheses. One approach is to establish controlled rheological conditions, without thrombus formation, and to measure the chemical effects. The other approach is to try to measure adenine nucleotides present during the formation of a thrombus. Neither approach serves to test the last hypothesis mentioned above.

Controlled rheological conditions have demonstrated the deformability of red cells under steady shear. The combination of normal and shear stresses serve to elongate the cell and make the membrane rotate around the cell contents in a tank-tread motion (Fischer *et al.* 1978). The concept that the red cell may act as a mechanoreceptor for shear more effectively then can the platelet itself is tied to the simple rheological fact that in a given shear flow the stresses induced around the red cell are much greater because of its larger size. After experience with microporous tubing in a hybrid artificial organ (Chick *et al.* 1975) in which ultrafiltration through

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the tube wall was significant, an experiment was developed in which a red cell suspension was pumped steadily through the lumen of a microporous tube and the ultrafiltrate that passed through the tube wall was collected for chemical analysis (Schmid-Schönbein *et al.* 1979). This ultrafiltrate was drawn from the region in the tube flow where the red cells were under the highest shear and should therefore contain any adenine nucleotides leaked from the red cells. It proved difficult to demonstrate whether the concentration of ADP in the ultrafiltrate was greater than that associated with the slight degree of haemolysis that occurred even when the maximum shear in the tube was below that required for haemolysis. In experiments that take the alternative approach of looking for adenine nucleotides effluent during the formation of a thrombus, Born & Kratzer (1981) have used the luciferin–luciferase system to identify ATP (and also by the use of pyruvate kinase, ADP) in the effluent from a freshly cut artery and a freshly punctured tube with blood flowing through. The early post-cut effluents do not show the presence of impressive amounts of nucleotides.

It has become clear that experiments to test the hypothesis about the nature of the chemical response of red cells under shear are difficult to perform because the amounts of adenine nucleotides being looked for are rather small. The results do not yet appear definitive.

There is an additional aspect of the experiment of Born *et al.* (1976) that merits consideration. This aspect is that there is very little time for the reaction to occur. It is possible to estimate for a typical experiment the extent over which there is an increase in wall shear in the region of the tube immediately upstream from the puncture: this is about 65 μm, and cells moving with their centres 2.5 μm from the wall will reach the lip of the hole in less than 25 ms, and (if there is no local region with closed streamlines formed) will take less than 1 ms in transit through the puncture. These times are short compared with the activation delay times discussed earlier. The experiments to determine the activation delay times were conducted at relatively low shear conditions, and it is possible that potentiation in flows at relatively high shear (as in the cut artery) may reduce the activation time. However, the potentiation and adhesive reactions have very little time to occur before the cells are swept away from the site of the cut, an aspect which may add weight to the hypothesis that the chemical effects involve the cell membranes rather than intracellular substances because the membranes are more readily available to act in such short times.

The extent of damage to platelets is also affected by the duration of the stress. Colantuoni *et al.* (1977) reported that with exposure times of the order of 10^{-3} s there were no significant changes measured in the platelets for stresses below 0.07 N/cm², that higher stresses lead to monotonic increases in plasma serotonin and that stresses above 0.15 N/cm² lead to diminution of the platelet count and to plasma LDH activity, interpreted as being due to lysis. For shorter exposure times, 10^{-4} s, stresses of the order of 0.4 N/cm² are needed to produce evidence of changes in platelets.

Drugs can affect the response of platelets to mechanical stress. For example, Hardwick *et al.* (1980) have shown that incubation of platelets with prostaglandin E_1 and theophylline before exposure to mechanical stress reduced shear-induced aggregation, but above roughly 1 mN/cm² there was increased serotonin release and lysis compared with controls. Effects of shear on leucocytes are less well known. McIntire *et al.* (1980) have shown that levels of shear 1–3 mN/cm² cause development of phagocytic vacuoles and loss of cytoplasmic granules in PMN leucocytes; they also found that incubation with 3 μM prostaglandin E_1 and 1 μM RA233 (a phosphodiesterase inhibitor related to dipyridamole) for 10 min before shear greatly reduced the mechanical damage suffered.

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6. Interactions between individual cells and walls

So far this review of rheological factors in cell – vessel wall reactions has concentrated on events surrounding thrombus formation because of the significance of the phenomenon. It is important to stress that most interactions between platelets (and red cells) and walls do not result in thrombus formation and consist of transient osculations rather than adhesion. Rheologists have known for some time that flow of slurries – dense suspensions of particles in fluids – often occurs with a relatively particle-free layer of fluid, the skimming layer, close to the wall in tubes with diameters many times the particle size. This phenomenon occurs also with blood flows. This might be thought to reduce or eliminate blood cell–wall contacts, but the skimming layer is a macrorheological phenomenon and at the microrheological level one finds frequent osculatory collisions between blood cells and the walls, and these collisions can be accompanied by exchange processes, as demonstrated by uptake of radioactive labels from the wall by red cells (Keller & Yum 1970). These collisions can therefore alter cells but usually in minor ways, and the changes are not like the all-or-nothing changes associated with platelet involvement in thrombus formation. The diffusion of cells to and from a wall is affected by the augmentation process described in §4.

Evidence is now growing that degrees of reaction can occur between platelets and walls that are intermediate between simple collision and permanent adhesion and aggregation. Richardson *et al.* (1979) have studied the adhesion of platelets to foreign surfaces and have observed that many platelets depart after adhesions lasting 2–3 min, and moreover that sites previously occupied by platelets were preferred for adhesion by platelets (passing subsequently) compared with unused adhesion sites. Adams & Feuerstein (1980) observed fluorescently labelled platelets in shear flow over a wall and reported that there were transient adhesions lasting up to 0.5 s, but that platelets adhering longer than that tended to remain for a relatively long time. Richardson *et al.* (1979) have observed that individual platelets that adhere and then come free often adhere again downstream, and that circumstances can prevail with this turnover process that the platelet adhesion density (platelets per unit area) becomes progressively larger downstream, suggesting that platelets can become more adhesive as they progress downstream after adhesion–detachment events. It is not clear whether repeated adhesions and detachments are necessary to augment the adhesiveness, or whether it occurs with the passage of time after a damaging event as illustrated by the results of Feuerstein *et al.* (1980) for serotonin release from the mechanically injured platelet.

A significant factor in controlling the interaction between individual cells and walls is the existence of an ultrafiltration flow through the wall. Forstrum *et al.* (1975) investigated formed element deposition onto porous walls for red cells and for platelets when ultrafiltrate leaves the blood stream. Noting that cells would experience a drag force towards a surface because of the ultrafiltration, they showed that when the value of a deposition parameter

$$\lambda \nu^{\frac{1}{2}} U_{\mathrm{f}} / R^2 S^{\frac{3}{2}}$$

(where U_{f} is the ultrafiltration velocity, λ is a function of the particle volume concentration, R is the particle radius, ν is the kinematic viscosity of the suspending phase and S is the wall shear rate) exceeds a certain value, cells will deposit at the wall even when there is no adhesion. For red cells the critical value of the parameter is about 0.15 and for platelets the critical value (which showed experimental scatter) ranged from 0.01 to 0.15. A corollary of this is that if

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ultrafiltration occurs through a wall in the opposite direction, i.e. into the bloodstream, it can prevent adhesion to a surface to which platelets would otherwise adhere, as demonstrated by Predecki *et al.* (1980*a*).

7. Flows around obstacles, in cavities, and into holes

Mural obstacles and cavities, such as those formed when atheromatous plaques fracture, assist thrombus formation. There are flow-controlled effects on lipid deposition processes near blood vessel bifurcations. These observations have led to interest in flows around obstacles, in cavities, and into holes branching from a vessel or duct. The experiments of Born *et al.* (1976), where a cut or hole is made in the side of a tube, provide another example of the latter type of flow. Unfortunately the flows are rather complicated, with streamlines taking paths that are sometimes very tortuous.

Baker (1979) has shown that a small cylindrical mural obstacle alters the structure of a simple shear flow by inducing formation of horseshoe-shaped vortex structures with the curved part of the shoe draped around the upstream side of the obstacle. These flows increase the residence time near the obstacle of the fluid entrained in these structures. This may help to provide time for fibrin polymerization in slow flows, or retention of platelets to times beyond their activation-delay times in faster flows. In any case, the experimental evidence shows clearly that the disturbance created is sufficient to enhance adhesion and thrombus formation. Flows in cavities have a large degree of recirculation so that residence times can be very long indeed. Flows at tube junctions have been studied with ranges of values of the Reynolds number, ratios of branch diameter to main tube diameter, ratios of branch flow rates to main supply flow rates, branch angles and so on. Rodkiewicz & Roussel (1973) have investigated conditions for large arteries with experimental Reynolds numbers in the range 700–5000, for example, and Karino & Goldsmith (1980) those for smaller Reynolds numbers (10–350). Both groups observed that the flow down the side branch had a double-helicoidal structure (reminding one of the secondary motion in curved tube flow) and that there were local regions of flow separation and reattachment. Of the flow which enters the side branch, some turns directly from the main flow while another fraction of it overshoots and then executes a 270° turn to reach the side branch rather like road traffic using a clover-leaf turn to gain access from one highway to another. Karino & Goldsmith found that the radius of curvature of the wall surface at the junction of the branch to the main tube had a profound effect on the extent to which separated flow zones were generated, with the smoother, more curved transitions generating less flow separation. The flow structures look vortical in places, such as the cloverleaf turn, but are open streamline flows and do not markedly increase residence times for most of the flow.

The studies mentioned above were carred out with steady flows in stiff-walled chambers. Some differences in the flows occur especially if the flow is pulsatile, and to some degree if the walls are compliant. Even without the modifications associated with such changes in conditions, the flow is complex enough to negate the prospects of simple analysis, but its description helps to provice a background against which transport of platelets to local portions of the wall can be compared.

8. Role of plasma proteins

It is well known that the rheology of whole blood is influenced by plasma proteins; most particularly, the viscosity of blood in steady shear flow is affected by the concentration of

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fibrinogen. The possible rheological role of plasma proteins in thrombosis has ben overwhelmed by their biochemical role, as fibrinogen is an essential constituent in platelet aggregation. On foreign surfaces the adhesion of platelets is mediated through plasma proteins that have adsorbed to the surface, and it would seem that to some degree the thrombogenicity of a surface is related to the degree to which it binds fibrinogen, and its passivity is related to the binding of albumin.

The alignment of long molecules such as proteins in a shear flow involves time constants for

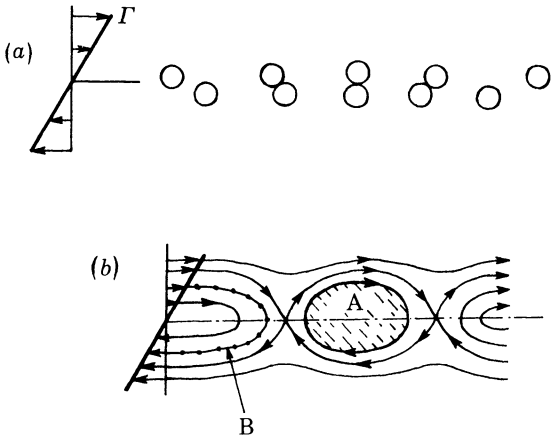

FIGURE 3. Cell–cell interactions. (*a*) Five successive views of two cells undergoing collision in shear flow with velocity gradient Γ. The rotation rate of cells is approximately $\frac{1}{2}\Gamma$. (*b*) Flow induced by red cell A in a shear flow. The points on streamline B show a relatively small particle or cell at successive intervals of time and illustrate how the flow induced by A can cause augmentation of the diffusion of particles across the mid-plane (shown as chain line).

relaxation, and in a pulsatile flow there is some question as to the extent to which the relaxation has occurred and therefore how much the long molecules affect the flow.

9. Discussion and conclusions

In most flows of practical interest for platelet–vessel wall interactions the cell–cell encounters are caused dominantly by shear-flow encounters rather than by Brownian motion. The fraction of collisions leading to aggregation is known as the collision efficiency; for platelets this is affected by the shear rate and by the degree of activation such as that caused by ADP. The apparent collision efficiency of platelets may be enhanced by the morphological changes (development of pseudopodia) that follow activation, as these morphological changes may increase the effective radius of the platelets. Red cells, which are of course larger than platelets, induce flows around themselves when they are in shear flows, and a consequence is to cause a considerable enhancement of platelet diffusivity in the direction of the velocity gradient. This effect increases with haematocrit when the haematocrit is very small, but is only weakly dependent on haematocrit if at all at normal physiological values. There is some possibility that the red cells serve as mechanoreceptors for increases in shear rates; such increases occur near sites of vascular injury. The response of the cells, through mechanical deformation, may be to provide a chemical signal sensitizing the platelets and leading to enhanced aggregation. This effect is not certain but has been proposed as the explanation of an oft-repeated experimental phenomenon. Cell–cell interactions are illustrated in figure 3.

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The interaction of platelets with vessel walls can involve simple collision, collision with some transient adsorption or adhesion with some mutual changes occurring between platelet and wall, or relatively permanent adhesion. Transient interactions, while involving changes in platelets, do not have activation of the all-or-nothing sense associated with them. Platelet–wall interactions can often be characterized in terms of a rate constant. Cell–wall interactions are affected by ultrafiltration through the wall, ultrafiltration from the blood stream being

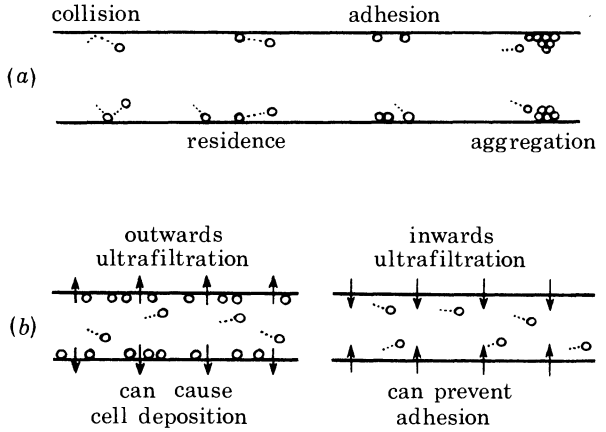

FIGURE 4. Platelet–wall interactions. (*a*) Successive illustration of a simple collision, residence (where collision is followed by a generally brief period of residence of the platelet with subsequent departure), adhesion (from which the platelet does not depart) and aggregation. (*b*) Effects of ultrafiltration through vessel walls.

capable of causing cell deposition without adhesion while ultrafiltration into the bloodstream is capable of preventing adhesion. Platelet–wall interactions are illustrated in figure 4.

The magnitude of the forces that can be exerted between platelets and a wall is not well known, although this is obviously important in determining the stability of adhesion under a pulsating flow and the maximum shear rate in which specific aggregates can be safe from shear-driven embolization. It does appear that there is an activation time delay for platelets to be able to exert sufficient force to sustain adhesion; this is a fraction of a second.

It has proved possible to develop a rheological theory for the growth of small mural thrombi (typically 50–100 μm long) that predicts the exponential growth of volume with time found in experiment and which relates the exponential coefficient to the shear rate in a way that corresponds with experiment when the effects of the activation delay time are taken into account. For long mural thrombi (more than 1 mm) it has been found that the growth rate can be limited by either the surface reactivity for platelets or the diffusion of platelets from the bloodstream, the latter being affected by the enhancement provided by red cells in a shear flow, as noted earlier.

Timescales have shown themselves to be important. On the one hand, at short times, there are the effects of the platelet activation delay time in limiting thrombus growth rates at high shear rates, and the evidence that the haemostatic reaction for vessel damage and for the experiment of Born *et al.* (1976) occurs in a small fraction of a second. On the other hand, additional time is provided for reactions to occur by bumps on walls and by cavities due to the fluid motions associated with these wall geometries. These extra-time situations are illustrated in figure 5.

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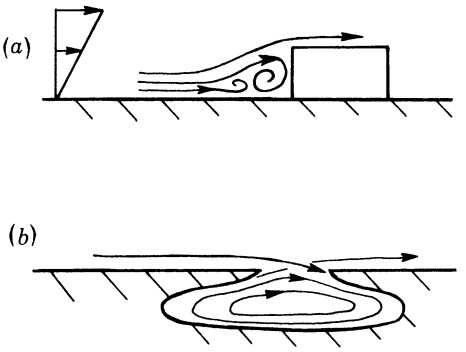

FIGURE 5. Situations leading to additional time being spent by portions of a blood stream in local regions near a surface. (*a*) Flow near an excrescence by which vortices are formed. The vortices drape themselves around the obstacle in the form of a horseshoe when viewed from above, and particles or cells entering each vortex are eventually swept downstream. (*b*) Flow over and in a cavity. While there is some exchange of fluid between the stream and the cavity, the residence time in the cavity is relatively long.

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Discussion

D. B. Longmore (*National Heart Hospital, London, U.K.*). The results presented in this important paper possibly may not reflect what happens in life. Studies with clear fluids in artificial systems fail to take into account two fundamental factors.

First, blood is an extremely complex non-Newtonian fluid. Its mechanical properties change with the physical environment and with flow patterns. It has been rightly pointed out that when blood is flowing above a critical velocity in a vessel, there is an axial stream of cells. The blood in contact with the vessel walls consists mainly of plasma, which consists in part of a range of plasma protein molecules. Some of these plasma proteins are highly deformable if stressed or put in shear. The normally barrel-shaped coiled molecules can deform to become long spirals or nearly straight. There is a profound change in viscosity when this occurs. No artificial blood has yet been made with these unique properties. Furthermore, when whole blood is pumped through artificial circuits the plasma proteins soon lose these properties.

The second important difference between any experimental model and life relates to the development of the cardiovascular system in the embryo. The mesoderm forms the cardiovascular system and the blood from one material. During the development of the embryo, the central liquifying blood-forming elements ebb and flow, and then flow through the vascular plexus, which is to form the adult tree. The detailed internal architecture of the vascular system is therefore at least partly moulded by the very blood that is flowing through it. As the foetus develops, the network of vessels soon develops into a definitive vascular tree possibly

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because of the fluid logistics of the system. Small biasing streams in a network deflect the whole flow in one direction.

Because blood has variable physical properties and it flows through vessels moulded by its flow, does Dr Richardson think it likely that we will obtain most information from studies of blood flow *in vivo*? Range gated pulsed Doppler instruments can show us more than just the anatomy of vessels, the flow rates and the velocity profiles within them. They can detect the difference between laminar flow and energy consuming turbulent flow.

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P. D. Richardson. I wish to thank Mr Longmore for his discussion, in which he has brought out five main points: the non-Newtonian rheological properties of blood, the role of plasma proteins, axial streaming of cells in tube flows, embryonic blood flow, and measurement of velocity profiles in whole blood flow. These points I should like to address briefly.

The non-Newtonian rheological properties of blood have been well documented, and the higher values of effective viscosity at smaller values of the shear rate are attributed to the aggregation of red cells into rouleaux. It is expected that there are larger rouleax near the centre of a tube carrying a blood flow than there are near the wall, because the shear stress is smaller near the centre than it is near the wall. Recently we have been able to demonstrate this by ultrasound (Abts *et al.* 1979). There is, however, a finite timescale for aggregation of rouleaux and also for disaggregation of rouleaux upon experiencing higher shear. Consequently some differences may be found between the bulk rheological properties of blood in steady flows and in flows with oscillations with time scales of the order of (or less than) the rouleaux relaxation timescales. As Mr Longmore points out, long-chain molecules can cause non-Newtonian properties in a fluid, and indeed these can be found with very dilute suspensions; however, here again there are characteristic timescales associated with the alignment of the long chains in a shear flow and with stretching of coils, and the rheological response at high enough frequencies of oscillation can be different from that in steady flows. Not enough is yet known about the quantitative details of the constitutive equations that represent this behaviour for blood. Mr Longmore is correct to observe that a coloured Newtonian liquid is not a rheologically perfect substitute for blood. It is only fair to point out that blood is not a simple fluid; even with a patient who undergoes heart–lung bypass as an adjunct to surgery, the blood in his circulation may start with a haematocrit of more than 40 % but can be reduced by haemodilution to 25 %, cooled, and partly depleted of plasma proteins by passage through the extracorporeal circuit, not to mention other changes, so that substantial alterations in the rheological properties can occur even during such a procedure. Changes also occur during life, and there are significant differences for different species.

Axial streaming is an interesting phenomenon, and there is not room here to treat it broadly. It should be noted, however, that the 'cell-poor' layer (or 'skimming' layer) near the wall is affected by axial development length, vessel curvature and pulsatility of the flow. Its relative significance depends on the vessel size.

Embryonic blood flow is a fascinating subject. Physiologists and biomechanicians alike are very conscious of the importance of convective transport of nutrients to tissue masses too large to be supplied adequately by diffusion alone from their peripheries, and the development of

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embryonic blood flow is interesting for this and for other reasons. The development of pulsatile action in the embryonic heart, even before the chambers are distinctly formed, may be important for augmenting transport because pulsatile motions can induce steady secondary motions that provide a vehicle for convection. Platelet – vessel wall interactions are poorly known in these circumstances.

In his final point, Mr Longmore raises the question of measurement of velocity profiles in whole blood flow. I agree wholeheartedly with his view that one should try to obtain measurements with real blood rather than with a substitute liquid. There are serious problems in obtaining accurate measurements in whole blood, especially if accurate measurements of velocity gradients are desired. Invasive measurements (e.g. hot-film anemometry) are not widely suited to different vessels; optical methods such as laser velocimetry run into problems as the vessel size rises above 50 μm or so because of absorption and scattering from the red cells, although it is possible to achieve good spatial resolution (Melling *et al.* 1976). Pulsed Doppler ultrasound has the ability to obtain measurements in larger blood vessels but generally lacks the spatial resolution to determine accurately the velocity gradient in the important region close to the wall. The incidence and structure of turbulence are somewhat hard to measure in human physiological flows because turbulence can be spatially patchy and temporally cyclic in large vessels and in extracorporeal circuits. This obviously complicates the platelet – vessel wall interactions.

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