Shear Stress Activation of Platelet Glycoprotein IIb/IIIa Plus von Willebrand Factor Causes Aggregation: Filter Blockage and the Long Bleeding Time in von Willebrand’s Disease

By J.R. O’Brien and G.P. Salmon

The article explores the finding that high shear alone applied to normal, native blood results in platelet aggregation. A filter with tortuous capillary-sized channels permits a study of the effect of shearing forces at different pressures. Native, heparinized, citrated and EDTA blood and platelet-rich plasma (PRP) were forced through the filter. Normal and von Willebrand’s blood were studied, as were the effects of antibodies to platelet glycoproteins (GPs) and to von Willebrand’s factor (vWF) and of “membrane-active” drugs. Normally, the filter blocked at 40 mmHg but not at 5 mmHg. Transmission electronmicroscopy of the filter at 40 mmHg showed blockage by platelet aggregates. Initially, the mean transit time through the filter was 8 milliseconds. Platelet retention in the filter occurred in two phases. From 0 to 3 seconds, only high-shear, vWF, and GPIIb/IIIa were required. From 10 to 20 seconds, retention presumably involved these three attributes, but divalent cations were also essential. Only this phase was inhibited by some membrane-active drugs. ADP- and thrombin-induced aggregation requires GPIIb/IIIa and fibrinogen. Shear-induced blocking of the filter by blood with a normal concentration of fibrinogen requires GPIIb/IIIa and vWF. This indicates a different type of exposure of GPIIb/IIIa. The long bleeding time in vWF disease highlights the absolute requirement for vWF and emphasizes the difference in exposure of GPIIb/IIIa induced by shear stress. Evidently, a process similar to that occurring in the filter is required in normal capillary hemostasis.

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The long template bleeding time at 40 mmHg in von Willebrand’s disease (vWD) is not fully understood. Ristocetin apart, vWF platelets behave normally in most tests, but abnormalities are regularly reported in conditions of high shear.1,2 This article explores the paradox that with high shearing forces, when it must be difficult to secure hemostasis, von Willebrand’s factor (vWF) is required. At low pressure, evidently other mechanisms are involved.

If native blood is forced through a polycarbonate Nuclepore filter with 5-μm holes, only a few single platelets adhere to the upper surface. Thus, the material is relatively inert. On the underside, massive clumps of platelets are deposited.3,4 Sheared platelets will aggregate and block small holes.5,6 A thin filter with tortuous capillary-sized channels permitted a detailed study of the effects of the shearing forces necessary to produce platelet aggregates that are retained and block the filter.

MATERIALS AND METHODS

The device is shown diagrammatically in Fig 1. The commercial filter is made of glass fibers 0.1 to 3.4 μm in diameter; they run in all directions (Fig 2), creating tortuous channels of varying geometry through the filter. Particles >10 μm in diameter are retained. The filter is made of glass fibers 0.1 to 3.4 μm in diameter; they run in all directions. From 0 to 3 seconds, only high-shear, vWF, and GPIIb/IIIa were required. From 10 to 20 seconds, retention presumably involved these three attributes, but divalent cations were also essential. Only this phase was inhibited by some membrane-active drugs. ADP- and thrombin-induced aggregation requires GPIIb/IIIa and fibrinogen. Shear-induced blocking of the filter by blood with a normal concentration of fibrinogen requires GPIIb/IIIa and vWF. This indicates a different type of exposure of GPIIb/IIIa. The long bleeding time in vWF disease highlights the absolute requirement for vWF and emphasizes the difference in exposure of GPIIb/IIIa induced by shear stress. Evidently, a process similar to that occurring in the filter is required in normal capillary hemostasis.

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FILTER BLOCKAGE AND LONG BLEEDING TIME IN von WILLEBRAND'S DISEASE

Chemicals and Antibodies

The following chemicals were obtained from Sigma, Poole, England: aspirin, apyrase (grade VIII), chlorpromazine, and chloroquine. Mepacrine was obtained from Boots, Nottingham, England, and cocaine was obtained from May and Baker, Dagenham, England. Stypven (Russell viper venom) and prostacyclin (PGI₂) were kindly presented by the Wellcome Foundation (Dartford and Beckenham, England). The antibodies to platelets GP1b and IIb/IIIa were kindly supplied by Drs B.S. Coller and W. Nichols and the antibodies to vWF were supplied by Dr E.J.W. Bowie. The kit for the platelet factor 4 assay was obtained from Abbott Diagnostics, Wokingham, Berks, England. Cryoprecipitate was obtained from the National Blood Transfusion Service. It was selected to have no red cell agglutinins, warmed to 37°C, passed through a standard filter in the apparatus to remove any particles, and then added to blood warmed to 37°C.

Patients

Twenty patients with severe atherosclerosis were studied seven times in 3 months. The correlations presented are derived from comparisons of the mean value for each individual. (Less complete data on normal subjects were similar).

Sixteen vWd patients were repeatedly examined. They were considered to be types I, IIa, and B, with factor VIIIc, factor VIIIcAg, and RCoF from 0% to 40%. Multimeric analysis was not performed.

Statistics

The degree of correlation was measured by the product moment correlation coefficient (r). Reproducibility: the relative variability of samples was compared using Karl Pearson’s coefficient of variation (CV).

RESULTS

Reproducibility

Within sample. Four aliquots from each of six people were tested at 40 mmHg. The mean CV of the drop count between 10 to 20 seconds was 9% ± 4%.

Within patient. Twenty patients with atherosclerosis were studied seven times over a 4-month period. The mean CV between these seven samples (10 to 20 seconds) was 20% ± 11%.

Fig 1. Diagrammatic representation of the device.

Fig 2. Scanning electronmicrograph of filter (original magnification x 1,800; current magnification x 810).

Fig 3. Cumulative drop count plotted against time. (1) Normal heparinized blood at 5 mmHg pressure. (2) Normal heparinized blood at 40 mmHg; shaded area indicates range (1 SD). (3) Normal heparinized PRP at 40 mmHg. (4) EDTA blood at 40 mmHg. (5) von Willebrand's blood. No blockage. (→); Filter "blocked" ( MediaPlayerThumb).
Between patients. The CV was established by comparing the mean of seven samples from each of the 20 patients: CV = 23%.
Platelet retention (10 to 20 seconds) figures were closely correlated with drop counts and had similar CVs.

Pressure
The crucial influence of different pressures was investigated first (Fig 3). Aliquots of the same heparinized normal blood were forced through the filter at different pressures. At 40 mmHg, blood initially passed rapidly through (14 ± 2 drops in 5 seconds; n = 40); within 20 seconds, however, marked slowing had occurred and on the average, the filter blocked in 34 ± 12 seconds (n = 40) when 214 × 10⁶ ± 62 (n = 40) platelets had been retained.

At 100 mmHg, initially on the average, 16 drops were forced through the filter but the tracing, platelet retention, and blocking were similar to those at 40 mmHg. At 5 mmHg (Fig 3), the initial rate was slow, but little decrease in the rate occurred (drop ratio 62 ± 20) and no blocking occurred in the filter.

Rate of Passage Through Filter

The volume within the filter was estimated by two methods. The filters were filled with water, and the increase in weight was 8 mg. The overall dimensions (9 mm in diameter; 0.26 mm thick) together with the makers' claim that one-half the volume was glass, gave a calculated volume of 8 mm³. Two milliliters EDTA PRP and vW PRP at 40 mmHg ran through in a continuous stream, and both took 2.1 seconds (n = 6). From this, the mean transit time can be calculated as 8 milliseconds. Normal heparinized PRP blocks the filter in 26 ± 7 seconds (n = 7). Before any platelets cause slowing, the transit time of normal PRP must be similar to that of EDTA or vW PRP. Thus, platelets exposed for 8 milliseconds to these shearing forces aggregate, accumulate, and finally block the filter.

Platelet Aggregation and Filter Blocking
Sections of the filter were prepared for TEM after 2, 8, and 20 seconds of flow, using normal blood or PRP at 5, 40, and 100 mmHg. Blood and PRP from vW patients, which do not block the filter, were also studied. At 40 mmHg, with normal PRP from 3 seconds on, increasing numbers of platelets were seen and the great majority of these had aggregated, with close apposition of the membranes (aggregation) and early centering of the granules is apparent. Original magnification ×29,160; current magnification ×14,000.

Cellular Damage
Cell damage could lead to leakage of aggregating agents from inside the cells. Effluent normal blood was centrifuged; the degree of hemolysis varied form 0 to, on occasions, 0.25%, but this did not correlate with blockage (n = 20). The plasma platelet factor 4 before passage was 10.6 ng/mL; after passage, the factor 4 was 26 ng/mL (n = 3). This increase would occur if 0.2% of platelets were lysed. The Stryven time of native blood showed little change (35 seconds before and 33 seconds after passage; n = 2). Thus, mechanical damage was minimal.

Calcium and Heparin
An absolute requirement for divalent cations was shown as follows. When native blood was tested at once, blocking occurred in 20 ± 6 seconds (n = 12). Aliquots (2.5-mL) of the same blood collected into citrate (final concentration 0.38%) did not block the filter but ran through in 33 ± 8 seconds (n = 5). Aliquots collected into liquid EDTA (final concentration 0.4%) poured through even faster (22 ± 7 seconds (n = 8)), and few platelets were retained (Fig 3, Table 1).

Blood collected into heparin (final concentration 4.0 IU/mL) blocked the filter in 34 ± 12 seconds (n = 40). Heparin, 20 IU/mL gave similar results.
WBC retention decreased to 22% ± 11% while the platelet retention increased (66% ± 15%).

The initial hemoglobin and the WBC count of blood correlated with, and so probably influenced, the initial drop rate (r = -0.35 and -0.41, respectively; n = 20), but they had no effect on platelet retention. The percentage of platelets retained in 0 to 3 seconds correlate with the percentage retained between 10 and 20 seconds (r = -0.82; n = 20). WBC retention 0 to 3 seconds did not, however, correlate with any other parameters; on the other hand, WBC retention (10 to 20 seconds), although low, tightly correlated with the platelet retention (10 to 20 seconds) (r = -0.70; n = 20).

vWF

Neither heparinized nor native blood from 16 patients blocked the filter at 40 mmHg; 2.5 mL blood ran through in 17 ± 4 seconds with little slowing (the drop ratio was 53 ± 7, and platelet and white cell retention was low) (Fig 3, Table 1). Even at 100 mmHg, there was equally little slowing; 2.5 mL ran through in 17 ± 8 seconds (n = 12).

Cryoprecipitate, 0.5 mL, added to 2 mL vWF blood raised the concentration of vWF from 27% ± 8% to 200% ± 6% (n = 5). Normal blocking occurred rapidly, with normal platelet (Table 1) and WBC retention.

Inhibition of Blocking (Table 2)

Inhibition of normal platelet retention and filter blocking was explored (a) by altering the temperature, since most enzymes are temperature-dependent; (b) by altering specific enzyme pathways; (c) by using specific antibodies to cover receptor sites on the platelets; and finally (d) by using nonspecific membrane-active drugs. All inhibitors were added 1 minute before testing.

Temperature. Heparinized blood collected in a room at 37°C and tested at once gave results similar to those obtained with aliquots incubated at room temperature for ≤1 hour (37°C: 33 ± 3 drops at 30 seconds; 22°C: 29 ± 4; n = 6).

Aspirin. When added in vitro, aspirin (10 mmol/L final concentration in distilled water) had no significant effect (Table 2). When patients swallowed 300 mg aspirin 2 hours before venepuncture, the filter results were similar to the pre-aspirin tests (n = 3).

Prostacyclin. At high concentration, prostacyclin (dissolved in 50 mmol/L Tris HCl buffer, pH 9.5) had no effect on platelet retention at 0 to 3 seconds, but significantly inhibited platelet but not WBC retention between 10 and 20 seconds. The addition of this buffer alone had no effect.

Apyrase. In the aggregometer, apyrase 1.7 U/mL, dissolved in distilled water abolished aggregation induced by ADP mol−2/L. In the filter, the addition of mol−2/L to heparinized normal blood at 37°C 1 minute before testing decreased the blockage time from 30 to 15 seconds (n = 3). The addition of apyrase before the ADP completely abolished the ADP effect. Thus, the apyrase was fully active. Added to normal heparinized blood, it had no effect on the filter results (n = 6).

Chloroquine. At a final concentration of 0.15 mmol/L, this drug, dissolved in distilled water, consistently promoted
rapid blocking and platelet retention increased from 65% to 91% (n = 3). At a concentration of 0.38 mmol/L, no effect was noted, but at 1.5 mmol/L platelet retention between 10 and 20 seconds was reduced to 30% of the control values and no blocking occurred.

Chlorpromazine, mepacrine, and cocaine. Relatively high concentrations of chlorpromazine, mepacrine, and cocaine dissolved in distilled water produced gross inhibition between 10 and 20 seconds, with results similar to chloroquine 1.5 mmol/L. The effect of lower concentrations was not explored.

**Inhibition of WBC retention (Table 2).** None of the inhibitors affected WBC retention between 0 and 3 seconds, but on every occasion between 10 and 20 seconds WBC retention was decreased by chlorpromazine and cocaine. Chloroquine, mepacrine, and PGI2 had no effect.

**Effect of antibodies to GPrIb, GPrIIb/IIIa, and vWf (Table 3).** Three antibodies to GPrIIb/IIIa were available; one to GPrIb was available. These were reconstituted in distilled water so that 0.1 mL added to 5 mL of normal, heparinized blood gave final concentrations of 5 to 13 μg/mL. These mixtures were incubated at room temperature for 1 minute. Controls consisted of 5-mL aliquots treated identically but with no additions. The order of testing was random. Each of the 10 major platelet aggregation proteins was available, and controls were given in Table 3. All three antibodies to GPrIIb/IIIa halved the platelet retention between 0 and 3 seconds, and two reduced retention 10 to 20 seconds to 20% and 22% of the control values. The third antibody, 7E3, was less active, but with all three antibodies there was little progressive decrease in the drop rate, ie, a high drop ratio. All three also inhibited WBC retention during the period of 0 to 3 and 10 to 20 seconds.

The antibody 6DI at a concentration of 2.7 μg/mL completely inhibited ristocetin (1.5 mg/mL)-induced aggregation of normal PRP. Thus, it was fully active. Mixed at a concentration of 13 μg/mL for 1 minute with blood at 20°C, it had at most a minor effect (Table 3).

Four antibodies to vWF were each studied five times. Fifty microliters of antibody added 1 minute before testing to 5 mL normal heparinized blood inhibited blocking and, on average, platelet retention 0 to 3 seconds and 10 to 20 seconds were decreased respectively by 24% and 45% of the controls (Table 3).

**DISCUSSION**

The main conclusions from these experiments are that the filter is blocked by platelets in two phases. The first, lasting 0 to 3 seconds, requires shear stress, vWf, and GPrIIb/IIIa. The second (10 to 20 seconds) has an absolute requirement for divalent cations. Stress, vWf, and GPrIIb/IIIa are apparently still required.

A global test in which whole blood is forced through capillary-sized holes was deliberately chosen to simulate, in part, the in vivo bleeding time, and it is unlikely to be influenced by a single mechanism. Because Apyrase and aspirin have no significant effect, ADP and thromboxane A2 are apparently not involved. Cyclic AMP may be (PGI2), but three phosphodiesterase inhibitors (theophylline, aminophylline, and PGE1; data not given) had no effect. Heparin and native blood results are similar, thus clotting and a heparin–platelet effect seem unlikely.

This is the first experimental situation that has allowed a precise estimate of the speed of shear-induced platelet aggregation.21,22 The initial mean transit time with normal PRP, and before aggregates obstruct the flow, is 8 milliseconds, yet in this time platelets are activated and aggregate. Subsequently, the filter is blocked when sufficient platelets have been retained, eg, 70% of platelets in 20 drops (1 mL blood) \(\approx 150 \times 10^6\) platelets.

The contribution of glass that can activate platelets, WBCs, and plasma seems excluded by the reported Nucleapore experiments.9,10 From the evidence presented, it appears

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**Table 2. Platelet and WBC Retention and Drop Ratio in the Presence of Inhibitors**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration</th>
<th>n</th>
<th>0-3 s</th>
<th>10-20 s</th>
<th>Drop Ratio</th>
<th>0-3 s</th>
<th>10-20 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>1.7 mmol/L</td>
<td>4</td>
<td>95</td>
<td>25*</td>
<td>172*</td>
<td>124</td>
<td>63</td>
</tr>
<tr>
<td>Cocaine</td>
<td>2.9 mmol/L</td>
<td>7</td>
<td>109</td>
<td>54*</td>
<td>156*</td>
<td>105</td>
<td>64</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>1.5 mmol/L</td>
<td>4</td>
<td>108</td>
<td>29*</td>
<td>153*</td>
<td>123</td>
<td>117</td>
</tr>
<tr>
<td>Mepacrine</td>
<td>1.0 mmol/L</td>
<td>4</td>
<td>86</td>
<td>26*</td>
<td>157*</td>
<td>131</td>
<td>130</td>
</tr>
<tr>
<td>PGI2</td>
<td>25 ng/mL</td>
<td>5</td>
<td>119</td>
<td>51*</td>
<td>157*</td>
<td>113</td>
<td>127</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10 mmol/L</td>
<td>4</td>
<td>—</td>
<td>91</td>
<td>108</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Apyrase</td>
<td>1.7 U/mL</td>
<td>5</td>
<td>125</td>
<td>114</td>
<td>78</td>
<td>88</td>
<td>100</td>
</tr>
</tbody>
</table>

Results are expressed as mean percentage relative to various control results. A low retention figure and a high drop ratio figure indicate inhibition.

\*p < 0.01

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**Table 3. Platelet Retention and Drop Ratio in Presence of Antibodies**

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Antibodies to GPrIIb/IIIa</th>
<th>n</th>
<th>0-3 s</th>
<th>10-20 s</th>
<th>Drop Ratio</th>
<th>0-3 s</th>
<th>10-20 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ES*</td>
<td></td>
<td>3</td>
<td>49</td>
<td>22</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP1/10*</td>
<td></td>
<td>4</td>
<td>61</td>
<td>20</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7E3*</td>
<td></td>
<td>2</td>
<td>53</td>
<td>54</td>
<td>111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody to GPrIb</td>
<td></td>
<td>6D1*</td>
<td>4</td>
<td>105</td>
<td>80</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Antibody to vWF</td>
<td></td>
<td>W1-3*</td>
<td>5</td>
<td>92</td>
<td>55</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>W1-5*</td>
<td>5</td>
<td>68</td>
<td>34</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>93E 4*</td>
<td>5</td>
<td>64</td>
<td>50</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>94H 5*</td>
<td>5</td>
<td>80</td>
<td>53</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as the mean percentage of the various control values.
unlikely that WBCs initiate blockage. Chlorpromazine and cocaine inhibit WBC retention and chloroquine and mepacrine do not, but all four agents inhibit platelet retention (Table 2).

Although RBCs have a profound effect on the initial flow rate, their removal in PRP does not impair filter blocking. Thus, ADP leaking from even intact RBCs is irrelevant.

Numerous authors report that platelet retention in glass bead columns and in vascular models is abnormal in vWD disease but only in situations of high shear. The absence of filter blocking in this model in vWD, its correction by the addition of cryoprecipitate, and the correction by DDAVP and the probable effect of high-mol-wt multimers of vWF all support the concept that vWF is essential in conditions of high shear.

With use of monoclonal antibodies, it has been shown that GPRIb/IIIa on the platelet membrane is necessary for filter blockage (Table 3) and that GPRIb is of little or no importance although it may be in other systems. In purified systems, GPRIb/IIIa has been reported to be activated, eg, by thrombin, at a site exposed with a conformational change before vWF can bind to the platelet. In these circumstances, however, if fibrinogen is present or added later, the fibrinogen will preferentially bind to the platelet surface or displace the vWF in fibrinogen. In the filter and in Baumgartner's models, normal fibrinogen levels are present, yet vWF is required. It follows that in stress-induced activation the expression of GPRIb/IIIa must differ from that which occurs when aggregating agents such as ADP and thrombin are added. In the aggregometer, vWF platelets behave normally after the addition of ADP, and the GPRIb/IIIa binds to the fibrinogen as the ligand. In the high-shear situation, both adhesive proteins, vWF and fibrinogen, may be required, but this does not alter the conclusion that high shear causes a unique expression of GPRIb/IIIa that requires vWF for platelet aggregation.

The differences in platelet retention between 0 to 3 seconds and 10 to 20 seconds show clearly that two processes are involved, although the events at 0 to 3 seconds (first phase) must also contribute to or even determine the events in the second phase (10 to 20 seconds) since they are closely correlated. There are important differences, however. Table 4 shows that phase 1 requires high shear, vWF together with "available" platelet membrane GPRIb/IIIa, but is independent of divalent cations (EDTA blood). Phase 2 is presumably also dependent on vWF and GPRIb/IIIa but differs from phase 1 in that it is inhibited in the absence of divalent cations and by the addition of membrane stabilising drugs.

Shear stress initially appears to expose GPRIb/IIIa in a way that is independent of fibrinogen and divalent cations. This locus with vWF permits platelet aggregation. Thereafter, the same or another vWF molecule becomes attached to GPRIb/IIIa through a cation-dependent bond, and only this second step is inhibited by perhaps a more general membrane perturbation caused by membrane-active drugs.

The effect of these membrane-active drugs, in admittedly high (mmol/L) concentrations, is not fully understood. The stimulating effect of lower concentrations of chloroquine is typical of some membrane-active drugs. Cocaine and quinine inhibit ADP- and thrombin-induced aggregation. Inhibition by local anesthetics of transmembrane calcium migration and the inhibition of filter blocking by EDTA may be related phenomena. Dibucaine and chlorpromazine are reported to influence membrane phospholipase A2. The inhibitory effect of PGI2 at 25 ng/mL may be due to a "membrane-stabilizing" effect, and PGI2 is reported to prevent the binding of vWF to platelet GPRIb. The lack of temperature dependence favors a conformational change rather than an enzyme effect. Finally, it must be pertinent that HPase, PGE, and dibucaine all inhibit the rate of binding of antibody to GPRIb/IIIa in both buffer- and ADP-stimulated platelets.

In conclusion, we propose, in agreement with Schmid-Schonbein et al that acute rapid bending of the platelet causes membrane perturbation and that this alone can, in milliseconds, induce a unique exposure of GPRIb/IIIa. Initially, only vWF is required. Seconds later, calcium is required for further platelet aggregation. Collagen, subintima, RBCs, and thrombin are not required for this type of aggregation, nor probably is fibrinogen or ADP. This is the first clear demonstration of a further independent way of inducing platelet aggregation. Accordingly, in patients with vWD, the long bleeding time at 40 mmHg results from an absence of vWF, which is essential for shear-induced platelet aggregation in this system and presumably in other systems. In contrast, ADP and thrombin involve a different expression of GPRIb/IIIa since aggregation then requires fibrinogen and vWF is irrelevant. Thus, ADP and thrombin apparently do not play a major part in determining the normal bleeding time. These findings presumably are relevant to hemostasis and thrombosis.

**ACKNOWLEDGMENT**

We thank Drs M.B. Zucker, B.S. Coller, and P.J. Green for valuable criticism; Drs B.S. Coller, E.J.W. Bowie, and W.L. Nichols for the gifts of antibodies; R. Griffin, FIMLT, for the electronmicroscopy; D. Ling for designing and constructing the device; Pall (Biomedicals), Portsmouth, England, for the filter sheets; S.A. Jamieson for extensive secretarial help; and the Portsmouth surgeons for permission to study the atherosclerotic patients.

**REFERENCES**


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**Table 4. Differences Between Platelet Retention During First Phase (0-3 s) and Second (10-20 s)**

<table>
<thead>
<tr>
<th>Addition</th>
<th>n</th>
<th>0-3 s</th>
<th>10-20 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil (normal)</td>
<td>40</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>vWF</td>
<td>16</td>
<td>56</td>
<td>36</td>
</tr>
<tr>
<td>Anti-GPRIb/IIIa</td>
<td>9</td>
<td>54</td>
<td>32</td>
</tr>
<tr>
<td>EDTA</td>
<td>8</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Membrane-active drugs</td>
<td>19</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td>PGI2</td>
<td>5</td>
<td>119</td>
<td>51</td>
</tr>
</tbody>
</table>

Average differences in platelet retention are expressed as a percentage of the appropriate normal values.


34. Graalnick HR, Williams SB, Coller BS: Fibrinogen competes with von Willebrand factor for binding to the glycoprotein IIb/IIIa complex when platelets are stimulated with thrombin. Blood 64:797, 1984


40. Vanderhoek JY, Feinstein MB: Local anaesthetics, chlorpro-
mazine and propranolol inhibit stimulus-activation of phospholipase A2 in human platelets. Mol Pharmacol 16:171, 1979


44. Meyer D, Baumgartner HR: Role of von Willebrand factor in platelet adhesion to the subendothelium. Br J Haematol 54:1, 1983


Shear stress activation of platelet glycoprotein IIb/IIIa plus von Willebrand factor causes aggregation: filter blockage and the long bleeding time in von Willebrand’s disease

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