Effects of Red Blood Cell Concentration on Hemostasis and Thrombus Formation in a Primate Model

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Because the effects of red blood cell (RBC) concentration on hemostasis and thrombus formation have not been studied experimentally under conditions of whole blood flow without anti-coagulation, normal baboons were bled or transfused to obtain three different groups: a low hematocrit (Ht) group (20% < Ht < 25%), a normal Ht group (35% < Ht < 40%), and a high Ht group (50% < Ht < 55%). Measurements of platelet count, bleeding time, platelet aggregation, fibrinogen level, and coagulation time (APTT) were equivalent to normal values in each group. Thrombus formation was induced using a device composed of collagen-coated tubing followed by two sequentially placed expansion chambers designed to exhibit flow recirculation and stasis. The device was exposed for up to 40 minutes in an arterio-venous shunt system. Wall shear rates in the tubular collagen segment were 100 seconds⁻¹ and 500 to 750 seconds⁻¹. The accumulation of ¹¹¹In-platelets and ¹²⁵I-fibrinogen/fibrin was measured radioisotopically; RBC incorporation was determined from measurements of total thrombus hemoglobin.

Thrombus that formed on the collagen substrate was rich in platelets and poor in fibrin and RBCs. Under high flow conditions, thrombus composition showed no dependence on Ht. Surprisingly, under low flow conditions, platelet thrombus volume was negatively correlated with Ht (r = −.73, P = .005), and it was increased by greater than twofold in the low Ht group as compared with the high Ht group. Thrombus that formed in the disturbed flow regions contained relatively few platelets but was rich in fibrin and RBCs. The predominant finding was a positive correlation between RBC incorporation and Ht at both high and low shear rates (r = .90, P = .00003; and r = .77, P = .002, respectively), with thrombus volume increasing three- to sixfold between the low and high Ht groups. Thus, in vivo variations in Ht ranging between 20% and 55% did not affect hemostasis, but were found either to promote or inhibit the net accumulation of thrombus, depending on local flow conditions.

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R E D BLOOD CELLS (RBCs) are an important component of the hemostatic mechanism. In vitro, platelet adhesion on the subendothelium is virtually abolished in the absence of RBCs and increases as hematocrit (Ht) values increase.¹⁻⁴ In vivo, anemic patients may exhibit a prolongation of the bleeding time, which usually normalizes when Ht values exceed 30%.⁵⁻⁸ This effect has been principally attributed to changes in the rheologic properties of the blood when RBCs are present, i.e., an increase in platelet diffusivity due to RBC motions causing enhanced transport of platelets toward the vessel wall.⁹

The role of RBCs in thrombus formation has also been investigated. Studies with in vitro perfusion systems and anti-coagulated blood have shown that platelet thrombus formation increases with increasing RBC concentration, but only at high shear rates.¹¹⁻¹³ This effect could not be demonstrated at low shear rates because no thrombi formed under these conditions, regardless of the Ht value.³ In vivo, it has been suggested that the risk of developing thrombosis may be increased in patients with erythrocytosis, although clinical studies have given contradictory results in patients with coronary thrombosis¹⁰⁻¹⁴ and acute stroke.¹⁵⁻¹⁹

The aim of this study was to evaluate the effects of variable circulating RBC concentration on hemostasis and thrombosis in a primate model. Because this experimental in vivo system does not require the use of anti-coagulants, conditions associated with increased platelet deposition should also cause amplification of coagulation-related events at sites of forming thrombus.²⁰⁻²¹ Baboons were chosen for these studies because this species is hemostatically similar to humans,²² and because the animals may be infused with homologous RBCs without causing hemolysis or other adverse transfusion reactions. Thus, by selectively bleeding or transfusing animals, steady-state hematocrit levels ranging from 20% to 55% could be readily attained. The effect of RBC concentration on hemostasis was assessed by measurements of bleeding time. Thrombus formation was studied under conditions of variable blood shear rate and hematocrit using a thrombogenic device that generates both platelet-rich and fibrin-rich thrombus.²⁰ Using this system, thrombi formed under the different test conditions were characterized by measuring their relative contents of platelets, fibrin, and RBCs.

MATERIALS AND METHODS

Animal studies. Six normal healthy male baboons (Papio anubis/cynocephalus) weighing 9 to 11 kg were used. All procedures were approved by the Institutional Animal Care and Use Committee in accordance with federal guidelines (Guide for Care and Use of Laboratory Animals, 1985). As previously described,²⁰ all animals had an exteriorized shunt surgically placed between the femoral artery and vein. The shunts did not shorten platelet survival detectably or produce measurable platelet activation.²³

The animals were bled or transfused, respectively, to obtain three different groups with Ht values in the range 20% < Ht < 25% (group
APT values (33 removed by extensive washing.) The two chambers were constructed using Teflon tubing, 9.3-mm id, connected by a 1-cm length of silicone rubber tubing (3.2-mm id). The devices were exposed to native blood for up to 24 hours. Blood flow in the device was freely established and measured using a poral circuit consisted of two devices placed in parallel. In one branch, local flow alterations secondary to thrombus formation, or of the effects of pulsatility per se (v-steady flow), were considered beyond the scope of this study. Rather, each hematocrit group was evaluated under defined conditions of high and low initial mean blood flow rate as discussed above.

Platelet deposition was determined using autologous platelets labeled with 111In-oxine as previously described. The average labeling efficiency was greater than 85%. Platelet accumulation was measured using a Picker DC 4/11 Dyna scintillation camera (Picker Corp, Northford, CT) interfaced with a Medical Data Systems A1 image processing system (Medtronic, Ann Arbor, MD). Good spatial resolution was achieved by acquiring only the low (172 keV) energy peak of 111In with a 10% energy window and high sensitivity collimator. Dynamic images were acquired at 5-minute intervals over the study period. Immediately after each experiment, standards consisting of 3 mL of whole blood (blood standard) as well as an identical thrombogenic device filled with autologous blood (device standard) were imaged for 5 minutes. Deposited 111In-platelet activity was determined by counting the radioactivity in the regions corresponding to the collagen-coated tubing and to the chamber portions of the device, and subtracting the corresponding device standard activity for each region. The total number of deposited platelets (labeled plus unlabeled) was calculated by dividing the deposited platelet activity (cpm) by the blood standard platelet activity (cpm/mL) and multiplying by the circulating platelet count.

Blood was drawn and collected aseptically into acid-citrate-dextrose (1 part ACD to 5 parts blood), and then centrifuged at 1,000 × g for 5 minutes. The supernatant was discarded and the RBC fraction was washed twice in 5% dextrose. The concentrated RBCs were subsequently infused into the study animals. Measurements of hemostasis and thrombus formation in the different groups were assessed at least 12 hours after the last blood manipulation (bleeding or transfusion). Hemostasis was assessed by measurements of bleeding time performed on the shaved volar surface of the forearm using the standard template method.

Thrombus formation was assessed by determining the relative amounts of platelets, RBCs, and fibrin deposited within a thrombogenic device connected to the arterio-venous shunt as an extension segment. The device was composed of a 2-cm length, 3.2-mm inner diameter (id) tubing segment coated with type I collagen, followed by two sequential tubing segments of expanded inner diameter. The collagen was uniformly deposited and not removed by extensive washing. The two chambers were constructed using Teflon tubing, 9.3-mm id, connected by a 1-cm length of silicone rubber tubing (3.2-mm id). The devices were exposed to native blood for up to 40 minutes. In each experiment, the extracorporeal circuit consisted of two devices placed in parallel. In one branch, blood flow was freely established and measured using a Doppler ultrasonic flowmeter (L and M Electronics, Daly City, CA); initial blood flow rates ranged from 100 to 150 mL/min. In the other branch, blood flow was maintained at 20 mL/min using a peristaltic roller pump (Cole-Parmer, Model 7016, Chicago, IL) placed distal to the thrombogenic device. While blood flow in the device was laminar, the chamber regions were designed to produce a complex flow pattern typically exhibiting annular vortex formation, reverse flow along the wall, and a prolonged residence time of blood cells and procoagulant material.

This system caused the formation of complex thrombus that was both rich in platelets (collagen segment) and fibrin (expansion regions). The tubing diameters of the device components were chosen so that, over the range of available blood flow rates (20 to 150 mL/min), initial wall shear rates on the collagen segment would reflect values found in arteries (100 to 750 seconds⁻¹), while those in the expansion regions would be more typical of venous flow conditions (<10 seconds⁻¹ at 20 mL/min). At these tubing diameters, an expansion region length of 2 cm was determined from previous experimental observations showing that the overall ratio of deposited fibrinogen to platelets was seven times higher in the two expansion regions than on the collagen segment. The device was constructed of silicone rubber and Teflon tubing since both have been shown to be relatively nonthrombogenic in this primate shunt model.

Blood flow in both branches of the circuit was pulsatile. However, evaluation of the possible importance of differences in flow pulsatility between branches, local flow alterations secondary to thrombus formation, or of the effects of pulsatility per se (v-steady flow), were considered beyond the scope of this study. Rather, each hematocrit group was evaluated under defined conditions of high and low initial mean blood flow rate as discussed above.

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| Table 1. Blood and Hemostatic Parameters in the Animal Study Groups |
|------------------------|------------------------|------------------------|
|                       | Group I                | Group II               | Group III              |
| Ht (%)                | 22.5 ± 0.3             | 38.7 ± 0.8             | 51.7 ± 0.6             |
| RBC (× 10⁶/μL)        | 3.0 ± 0.1              | 5.2 ± 0.1              | 6.9 ± 0.1              |
| HGB (g/dL)            | 7.9 ± 0.1              | 13.0 ± 0.4             | 17.3 ± 0.2             |
| MCV (μm³)             | 74.9 ± 0.9             | 74.5 ± 0.4             | 75.1 ± 0.4             |
| MCH (pg)              | 26.3 ± 0.5             | 24.9 ± 0.2             | 25.3 ± 0.2             |
| WBC (× 10³/μL)        | 10.2 ± 1.6             | 9.1 ± 2.6              | 11.8 ± 2.4             |
| Platelets (× 10³/μL)  | 301.0 ± 30.0           | 290.0 ± 27.0           | 268.0 ± 28.0           |
| Agg (%)               | 70.0 ± 4.0             | 69.0 ± 4.0             | 69.0 ± 4.0             |
| BT (min)              | 4.0 ± 0.2              | 3.2 ± 0.2              | 3.3 ± 0.1              |
| Fg (g/L)              | 2.4 ± 0.2              | 2.5 ± 0.1              | 2.9 ± 0.1              |
| APTT (s)*             | 32.0 ± 1.0             | 36.0 ± 1.0             | 40.0 ± 1.0             |

*Whole blood for APTT determinations was anti-coagulated with a fixed volume of 3.8% citrate (9:1 vol/vol). Studies in three additional control animals with citrate/plasma ratios (0.143, 0.181, 0.230) that were the same as those in the low, intermediate, and high Ht groups, respectively, gave APTT values (33 ± 1 s, 36 ± 1 s, and 41 ± 2 s) which were equivalent to those obtained in groups I, II, and III, respectively, indicating that APTT values differed as a consequence of variable citrate dilution only. All values are mean ± 1 SE of n observations.
(platelets/mL) as measured in each experiment. Radioactivity values referred to platelet activity only; the plasma (nonplatelet) activity per milliliter of whole blood was calculated for each experiment by multiplying the activity per milliliter of plasma by the factor: (1-Ht). Plasma activities averaged 13.8% ± 1.6% of whole blood activities in these studies and were the same in the three study groups (13.9% ± 3.2%, 15.2% ± 0.7%, and 12.8% ± 3.0% in groups I, II, and III, respectively).

Fibrin deposition was determined using 125I-fibrinogen. Baboon fibrinogen was purified using a β-alanine precipitation procedure, and homologous fibrinogen preparations were labeled with 125I using the ICl method as described.20 Labeling efficiencies averaged 70%. The thrombin clottability of the labeled fibrinogen was greater than 90%. Five microcuries of 125I-fibrinogen was injected intravenously 10 minutes before device blood exposure. At the end of each study, the thrombogenic device was thoroughly washed with isotonic saline and then cut into segments containing the collagen-coated surface and chambers (regions).20 Total fibrin deposition was calculated by dividing the deposited fibrin activity (cpm) by the clottable plasma fibrinogen activity (cpm/mg) in samples taken immediately before each experiment as described.20,21 The 125I-emissions associated with the fibrinogen samples and graft components were determined after allowing 30 days for the 111In-radioactivity to decay (half-life: 2.8 days).

The number of RBCs deposited within each component of the thrombogenic device was also determined. The separate segments comprising each device were kept in distilled water at 4°C for 30 days. After determining the content of fibrin, thrombi were thoroughly mixed with the distilled water vehicle (2 to 4 mL) to ensure complete lysis of all RBCs, with the residual material consisting only of white fibrin strands. The hemoglobin concentration of the homogeneous mixture was then determined using an automated cell counter (System 9000, Baker Instrument Corp, Allentown, PA). The number of RBCs was calculated by multiplying the hemoglobin concentration (g/mL) by the clottable plasma fibrinogen activity (cpm/mg) in samples taken immediately before each experiment as described.20,21 The 125I-emissions associated with the fibrinogen samples and graft components were determined after allowing 30 days for the 111In-radioactivity to decay (half-life: 2.8 days).

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RESULTS

Blood counts and hemostatic parameters for the three study groups are given in Table 1. Ht values averaged 22.5% ± 0.3%, 38.7% ± 0.8%, and 51.7% ± 0.6% in groups I, II, and III, respectively. While RBC counts and total HGB varied in proportion to Ht, measurements of MCV and MCH were not different between the groups (P > .5). Other measured parameters were also equivalent between the three groups, including the platelet count, extent of platelet aggregation (Agg) in response to collagen, APTT, and fibrinogen level (Fg).

Hemostasis. Hemostasis was assessed by measurements of bleeding time (BT), with results given in Table 1. The average bleeding time value in the animals with normal Ht (group II) was 3.2 ± 0.2 minutes. Bleeding times were minimally prolonged (4.0 ± 0.2 minutes) in animals with low Ht as compared with the high (3.3 ± 0.1 minutes) and normal Ht groups (P < .05 in both cases), although bleeding times in all groups were within normal limits (range: 3.0 to 4.5 minutes).21,23,24

Effect of variable flow conditions on thrombus formation. Thrombus formation was assessed by determining the relative amounts of platelets, fibrin, and RBCs deposited within the thrombogenic device. The device was exposed to blood for a maximum of 40 minutes. Under high flow conditions, occlusive thrombus formation with flow stoppage predictably occurred within 30 to 40 minutes. Under low flow conditions, the device was exposed for 40 minutes; no occlusions were observed over this time period.

Platelet deposition onto the collagen segment increased linearly with time under both high and low flow conditions (Fig 1, A and B). However, the rate of deposition was markedly higher at high flow. For example, in the normal Ht group (group II) after 10 minutes of blood exposure, five times more platelets were deposited on the collagen at high flow (5.93 ± 1.17 × 10⁵ platelets) than at low flow (1.06 ± 0.18 × 10⁵ platelets). However, levels of fibrin deposition onto collagen measured after blood exposure were similar at both low flow (0.46 ± 0.03 mg at 40 minutes) and high flow (0.39 ± 0.08 mg at 30 minutes) (Fig 2, A and B). Thus, in the animals with normal Hts, the overall ratio of deposited fibrin to deposited platelets was two times higher at low flow than at high flow. Few RBCs were deposited onto collagen regardless of the blood flow rate (Fig 2).

As compared with the collagen segment, the chamber regions accumulated fewer platelets but more fibrin and RBCs (Figs 3 and 4). The total number of platelets deposited in the chambers was reduced modestly under low flow versus high flow conditions (Fig 3, A and B). Thus, in the studies with animals having normal Hts, the total platelet deposition after 30 minutes averaged 4.92 ± 0.35 × 10⁵ platelets at low flow, and 7.69 ± 0.95 × 10⁵ platelets at high flow (P > .10). Conversely, the accumulation of fibrin and RBCs in the chambers was 3 to 4 times higher at low flow than at high flow (Fig 4).

Effect of variable RBC concentration. Thrombus formed on collagen under high flow conditions was not influenced by variable RBC concentration; the rate of platelet accumula-
tion and the total amount of platelets, fibrin, and RBCs deposited onto the collagen at the end of the blood exposure period were equivalent in the three study groups (Figs 1A and 2A). In contrast, under low flow conditions, platelet deposition onto the collagen-coated segment decreased as the Ht value increased (Figs 1B and 2B). Thus, after 40 minutes of blood exposure, the number of platelets accumulated on the collagen substrate averaged $15.57 \pm 3.45 \times 10^8$ platelets and $7.03 \pm 1.77 \times 10^8$ platelets in the low Ht and high Ht groups, respectively ($P = .06$). Figure 5 shows the inverse relationship observed ($r = -.58, P = .04$) between Ht and the number of platelets deposited on collagen at the low blood flow rate.

A previously reported linear relationship between blood platelet count and platelet deposition after 40 minutes of blood exposure at low flow was also seen in the present study with animals from each Ht group (Fig 6). Platelet accumulation onto collagen at high flow was also positively

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**Fig 1.** Total platelet deposition onto collagen at high (A) and low (B) blood flow rates. The time course of platelet deposition is shown for animals having low Ht (○), normal Ht (■), and high Ht (●) values as given in Table 1. Data are mean ± 1 SE of observations in three (■) or five (○) animals.

**Fig 2.** Effect of Ht on platelet, fibrin, and RBC deposition onto collagen at high (A) and low (B) blood flow rates. Endpoint measurements were taken after blood exposure for 30 minutes (A) or 40 minutes (B), in animals having Ht values that were low (I), normal (II), or increased (III). Time course measurements of platelet deposition are given in Fig 1.
correlated with platelet count \( r = .69, P = .03 \). Therefore, it was important that all study groups exhibited comparable circulating platelet counts (Table 1). Under the assumption that platelet deposition onto collagen at low flow was linearly related to platelet count, the data comprising Fig 5 were normalized as previously described to correct for modest platelet count differences and to estimate values of platelet deposition that would have resulted had all animals exhibited a fixed platelet count equaling the overall group mean (286,000 platelets/μL). The empirical inverse relationship between platelet deposition and Ht was substantially improved after correcting for variable platelet count \( r = -.73, P = .005 \).

The formation of fibrin on collagen was also dependent on Ht under low flow conditions. Thus, the total amount of fibrin deposited on collagen at low flow was modestly higher at low Ht than at high Ht \( (0.66 \pm 0.08 \text{ mg} v 0.46 \pm 0.06 \text{ mg}, P = .07; \text{Fig 2}) \). While it is expected that plasma fibrinogen levels should directly influence fibrin formation, and perhaps blood cell accumulation, the fibrinogen level per se was not evaluated as an independent variable in these studies since plasma fibrinogen concentrations varied little between individual animals or animal groups (Table 1).

Platelet deposition in the chamber regions of flow expansion was not affected significantly by RBC concentration at either low flow \( (P > .10 \text{ at 40 minutes}) \) or high flow \( (P > .50 \text{ at 30 minutes}) \) (Fig 3), but was found to depend on platelet count. Platelet accumulation after 30 to 40 minutes was positively correlated with platelet count at both low flow \( (r = .64, P = .02) \) and high flow \( (r = .68, P = .03) \). Similarly, levels of fibrin deposition were comparable regardless of the Ht value (Fig 4). However, the RBC concentration...
markedly influenced the extent of RBC accumulation in the chambers as shown by positive linear relationships found between Ht and RBC deposition at both high flow (r = .90, P = .00003) and low flow (r = .77, P = .002) (Fig 7). As compared with the low Ht group, RBC deposition in the high Ht group was increased sixfold at high flow (2.75 ± 0.26 × 10⁹ v 0.44 ± 0.14 × 10⁹, P = .0001) and threefold at the lower flow rate (7.66 ± 1.14 × 10⁹ v 2.57 ± 0.44 × 10⁹, P = .006) (Fig 4).

DISCUSSION

Hemostasis. Hemostasis was assessed in animals with variable RBC concentrations by the measurement of bleeding times performed using the standard template method.³⁴ Bleeding times showed no dependence on Ht, averaging 3 to 4 minutes in the different study groups. These values were within the reported normal range for baboons.²¹,²³,²⁴,²⁷

It is well-established that the presence of adequate numbers of RBCs supports hemostatic plug formation in vivo. Early studies by Duke³ and Hellem et al.⁴ showed that patients with severe anemia also exhibited a prolonged bleeding time that could be corrected by RBC transfusion. Similar observations have been reported for anemic, uremic patients in which prolonged bleeding times were corrected by increasing Ht values above 25% to 30%.²³ In vitro, blood perfusion over subendothelium has also shown that platelet adhesion increases as Ht values increase between 10% and 70%, particularly at high shear rates.²³ These observations have been attributed to the physicochemical effects played by RBCs for promoting platelet transport and platelet-surface interactions.²³ Thus, due to their small size relative to RBCs, platelets may be concentrated in a peripheral layer at the blood-vessel wall interface under some circumstances, thereby increasing their interactions with subendothelial tissue components.²³ The enhancement of platelet adhesion is probably unrelated to the availability of RBC adenosine diphosphate (ADP) since platelet adhesion is normal in the presence of RBC ghosts in perfusion studies²³ and is unaffected by the presence of ADP-scavenging enzymes (eg, apyrase).³⁹

In contrast to previous clinical studies, we did not find an increased bleeding tendency at reduced Ht. However, in the studies with anemic patients, some individuals also exhibited a reduced platelet count.⁶ In uremia, there may be additional hemostatic defects unrelated to RBC concentration,³⁶,³¹ which may be corrected in part by therapies that increase plasma levels of factor VIII/von Willebrand factor (eg, desmopressin).³² In addition, our study baboons were only moderately anemic (Ht > 20%) with no other clinical or biologic abnormalities. Under these circumstances, Ht values in the ranges studied presumably were sufficient to effectively augment platelet transport and prevent abnormal bleeding.³³

Thrombosis. A clinical relationship between elevated Ht and thrombotic risk is generally admitted, although not clearly demonstrated. Clinical studies have yielded contradictory results regarding a possible correlation between erythrocytosis, angina pectoris, and the occurrence of coronary...
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thrombosis, or the role of Ht and hemodilution in acute stroke. While thromboembolic complications occur in polycythemia vera, this myeloproliferative disorder may be associated with other abnormalities such as thrombocytosis. In a recent review, Ht was not found to be a risk factor for thrombosis in these patients. This conclusion is also supported by the observation that patients with secondary polycythemia are known to be relatively free of vascular complications.

In our studies, the role of RBCs in thrombus formation was assessed by determining the relative incorporation of platelets, fibrin, and RBCs into thrombi produced under conditions of variable flow and Ht. A thrombogenic device containing a segment of collagen-coated tubing followed by two tubular expansions was exposed to native blood using an arterio-venous shunt system as described previously. In each experiment, two devices were placed in a parallel branching design. In one branch, blood flow was spontaneously established at rates between 100 and 150 mL/minute with initial wall shear rates in the collagen segment ranging from 500 to 750 seconds\(^{-1}\). In the other branch, flow was constantly maintained at 20 mL/minute using a roller pump; the initial wall shear rate at the collagen surface was 100 seconds\(^{-1}\). Flow past the collagen segment was unidirectional, while regions of flow expansion typically exhibit flow reversal near the wall with recirculation of blood cells and procoagulant material. As shown previously, this device generates a complex thrombus having components that are both platelet-rich (collagen segment) and fibrin-rich (chamber regions), and which may be selectively blocked by anti-platelet agents or anti-coagulating amounts of heparin.

In the normal Ht group, platelet deposition on the collagen-coated segment increased monotonically at both high and low flow rates, but the rate of platelet accumulation was reduced under low flow conditions. Thus, after 10 minutes of blood exposure, five times fewer platelets were deposited at low flow than at high flow (Fig 1). This result is in general agreement with data obtained in perfusion test systems where increased shear rates produced increased platelet adhesion as well as thrombus formation. In contrast, fibrin deposition was similar at both high and low flow rates (Fig 2). The shear-rate dependence of fibrin deposition, ie, decreasing fibrin deposition with increasing shear rate, has been previously demonstrated when subendothelium is exposed in an annular perfusion chamber. However, in our study fibrin deposition was measured as an endpoint value only, after 30 to 40 minutes of device blood exposure. It is conceivable that a shear-rate dependence of fibrin deposition might be demonstrable at the shorter exposure periods typically used in earlier studies (eg, 5 to 10 minutes). Overall, the ratio of total deposited fibrin to total deposited platelets was twice as high at low flow than at high flow, with relatively few RBCs deposited on collagen regardless of blood flow conditions.

The chamber regions of flow expansion produced a venous-type thrombus, ie, one that was rich in fibrin and RBCs. In animals with normal Hts, the overall ratio of deposited fibrin to deposited platelets was about eight times higher in the chambers than on collagen, a value comparable with results reported previously. Similarly, thrombus formed in the disturbed flow regions was approximately 65 times richer in RBCs. Under high flow conditions, the accumulation in thrombus of fibrin and RBCs was reduced three- to fourfold versus the studies performed at the lower shear rate. Interestingly, the extent of platelet deposition in the chambers was comparable at both high and low flow rates. In an earlier study using a similar flow geometry, Karino and Goldsmith also observed that initial platelet attachment on the wall of a tubular expansion showed little dependence on shear rate. Thrombus formation on collagen under high flow conditions was not affected by variations in Ht ranging from 20% to 55%. The accumulation of platelets, fibrin, and RBCs was equivalent in the three study groups (Figs 1A and 2A). In contrast, in previous studies performed under comparable flow conditions but with anti-coagulated blood and a short exposure period (5 minutes), both platelet adhesion and thrombus formation on subendothelium increased continuously as Ht values were increased from 10% to 70%. In our study, a good correlation was found between platelet deposition onto collagen and Ht after 5 minutes of blood exposure (r = .64, P = .04), but no such relationship was found at later time points. This result suggests that the effects of variable Ht may be less apparent at longer exposure times, ie, that platelet thrombus formation at low Ht may be only transiently reduced.

In contrast, under low flow conditions, platelet deposition onto collagen was decreased as Ht values increased. Previous in vitro perfusion studies performed at comparable low shear rates (50 and 200 seconds\(^{-1}\)) showed no thrombus formation after 10 minutes for Ht values between 10% and 70%; ie, while increased RBC numbers promoted increased platelet adhesion, this effect was not associated with enhanced
thrombus formation. In the present study, platelet thrombus formation at low flow was significantly reduced as the RBC concentration was increased, and in a manner that also depended strongly on the circulating platelet count (Fig 6). These findings were striking and probably not related to possible alterations in platelets or coagulation produced by the transfusion of washed RBCs in the normal Ht and high Ht groups. Indeed, platelet function, as assessed by measurements of bleeding time and platelet aggregation, was unchanged. Similarly, the measurements of APTT and fibrinogen level were normal in all study groups. Moreover, any changes in platelet reactivity caused by these procedures should have been more apparent in the studies at higher shear rates since, under these conditions, platelet adhesion is less diffusion-limited and more strongly governed by the intrinsic reactivity of platelets toward the substrate surface.

While our findings are consistent with the possibility that increasing Ht may simply impede platelet transport under certain circumstances, other explanations are possible. For example, increasing Ht may have promoted the growth of individual thrombi, which were less stable with time, such that the overall (surface-averaged) accumulation of platelets was reduced at higher Ht. At comparable flow rates, the wall shear stress undoubtedly varied between study groups due to the strong dependence of blood viscosity on Ht. Shear forces will also vary with time, increasing locally as the lumen narrows. Because the extent, rather than rate, of blood element accumulation was measured in these studies, differences in microembolization rates due to shear stress differences could have influenced our observations, at least in part, and may account for the observed reduction in net platelet accumulation on collagen at increased Ht (Fig 1B).

Alternatively, in our system the near-wall concentration of platelets may have been reduced for Ht values exceeding about 20%. For example, it has been shown that under some conditions the enhanced transport of small species may at first increase with Ht, then decrease as Ht values exceed 50% due to particle crowding. In addition, baboons have slightly smaller RBCs than humans (mean volume: 72 to 78 μm³). While RBC size is thought to be important in this context, the effects of such differences (vs studies with human blood) remain largely undefined. Finally, additional studies suggesting that the role of RBCs in platelet deposition is not an entirely direct and positive one (ie, higher platelet deposition at higher Ht), and that platelet adhesion does not necessarily parallel thrombus formation, have also been reported. Thus, Baumgartner and Muggli showed that perfusion of rabbit blood at 300 seconds⁻¹ caused platelet adhesion and thrombus formation to increase with increasing Ht up to a value of approximately 30% to 40%, and that at higher Ht, thrombus formation was abruptly reduced while adhesion continued to increase. Local effects, including inhibition of platelet deposition on areas adjacent to growing thrombi, have also been described by Baumgartner and Muggli and Sakariassen et al.

In the expansion chambers, platelet deposition was largely independent of both flow and Ht (Fig 3). Previously, Karino and Goldsmith showed that the number of platelets adhering in a tubular expansion after 3 minutes may increase with increasing Ht. Similarly, after a short blood exposure period (5 minutes) at high flow, we observed a good correlation between Ht and platelet deposition in the chamber regions of flow recirculation (r = .69, P = .03). At low flow, platelet deposition in the tubular expansions was modestly reduced at high Ht versus the results at low or normal Ht (Fig 3B), perhaps as a consequence of the reduced platelet deposition seen on the upstream collagen-coated segment (Fig 1B). Indeed, previous experiments have shown that platelet deposition in the regions of flow expansion depends in part on the activation of platelets produced by the proximal collagen segment.

Lastly, under high and low flow conditions, a positive correlation was found between Ht and RBC accumulation in the annular regions of flow recirculation. As compared with the low Ht group, RBC deposition at high Ht was increased sixfold at high flow (P = .0001), and threefold at low flow (P = .006). Thus, in low shear regions of flow recirculation, rouleaux formation, and stasis, increased numbers of RBCs may effectively augment the total thrombus volume.

In summary, this study in normal primates demonstrates that hemostasis is unimpaired for Ht values ranging between 20% and 55%. In contrast, the extent of thrombus formation was shown to depend strongly on Ht. However, variable RBC concentration was found to either promote or inhibit the incorporation of different blood elements into forming thrombus, depending on local flow conditions. These results may explain in part the discordant conclusions reached in some clinical studies regarding the role of RBCs in thrombus formation.

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