Infectious virus titer in virus samples from cells treated with IF at 3 to 30 U/ml was reduced 50- to 250-fold (Fig. 1); virus particle production as measured by transcriptase activity or N protein was inhibited by a maximum of tenfold.

Purified virus samples with the same amount of radioactivity were applied on gels and subjected to electrophoresis. The gels were then sliced and the incorporation of radioactive precursor in each fraction was quantified. Results show a selective inhibition of G and M protein of VSV derived from IF-treated cells. Virus samples with the same amount of radioactivity were also analyzed on sodium dodecyl sulfate polyacrylamide slab gels, and the incorporation of radioactive precursors were quantified by fluorography (*9*). Four proteins (G, N, NS, and M, respectively) were present in samples of virus not treated with IF; however, in virus from IF-treated cells, there was a marked inhibition in G and M protein. The migration of protein was similar in both of the samples (Fig. 2).

These findings suggested that in the presence of IF not only is the production of infectious virus particles inhibited, but also the production of noninfectious virus particles is disproportionately enhanced. The results in many respects resemble those previously reported (*2*) in IF-treated cells infected with MLV. We concluded that such findings are not limited to murine RNA tumor virus systems.

The inhibition of membrane-associated virus yields by treatment with low concentrations of IF may be closely related to functional abnormalities in the protein incorporated into the virion or to absence of specific protein in the noninfectious virions produced (*2*). Since many studies (*10*) have demonstrated that VSV particles with reduced amount of glycoproteins are low in infectivity, it is likely that at least some of the reduced infectivity of VSV particles produced by IF-treated cells may be due to the reduced amount of this protein incorporated into such particles. It is possible that induced changes which have been reported to occur in the plasma membrane of IF-treated cells (*11*) may account for the alteration in infectivity of both VSV and murine RNA tumor virus, since these viruses bud from the cell surface as a terminal step in the replication process.

The noninfectious particles produced by IF-treated cells appear to contain only 42*S* viral RNA; they do not interfere with the growth of wild-type VSV, nor do they produce IF in culture. They are, therefore, unlikely to be defective interfering forms of VSV. Such noninfectious forms may, however, play a role in the initiation of chronic infections by VSV in IF-treated cells (*12*).

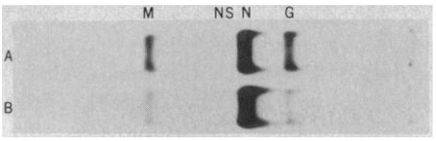

Fig. 2. Fluorograms of purified VSV proteins with equal amounts of radioactivity (15,000 count/min) applied to each gel: The gels were subjected to electrophoresis and then stained with Coomassie brilliant blue R. The gels were destained and impregnated with 2,5-diphenyloxazole (New England Nuclear), dried, and exposed to Kodak X-Omat x-ray film. Phosphorylase B (92,500 daltons), bovine serum albumin (69,000 daltons), ovalbumin (46,000 daltons), carbonic anhydrase (30,000 daltons), and cytochrome c (12,300 daltons) were used as reference protein molecular markers. (A) proteins of VSV released from cells not treated with IF; (B) proteins of VSV released from cells treated with IF (30 reference units per milliliter). *NS*, protein associated with the VSV transcriptase complex.

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Red Blood Cells: Their Dual Role in Thrombus Formation

Abstract. *Red blood cells may have a physical and chemical effect on the interaction between platelets and blood vessel surfaces. Under flow conditions in which primarily physical effects prevail, platelet adhesion increases fivefold as hematocrit values increase from 10 to 40 percent but undergoes no further increase from 40 to 70 percent, implying a saturation of the transport-enhancing capabilities of red cells. For flow conditions in which platelet-surface reactivity is more dominant, platelet adhesion and thrombus formation increase monotonically as hematocrit values increase from 10 to 70 percent. Thus red cells may have a significant influence on hemostasis and thrombosis; the nature of the effect is apparently related to the flow conditions.*

The accepted sequence of events in the formation of a platelet thrombus—platelet adhesion, release of platelet metabolites, and growth and stabilization of cellular masses consisting predominantly of platelets—generally attributes little role to the erythrocytes. However, abnormalities in red blood cell concentration in the absence of a demonstrated platelet abnormality can result in prolonged bleeding that is correctable by transfusion of red cells (*1*).

In flowing blood, overall platelet interaction with the subendothelium depends on two independent mechanisms: (i) transport of platelets from the blood to the vessel wall and (ii) reaction of the platelets with the vascular components. Platelet transport is governed by purely physical factors, namely the platelet diffusion coefficient and the blood shear rate at the vessel wall. Platelet-subendothelium reactivity is predominantly determined by chemical factors. Hellem (*2*) and Gaarder *et al.* (*3*) originally proposed that red cells play a chemical role by releasing adenosine diphosphate (ADP), a potent platelet-aggregating agent. Conversely, Turitto and Baumgartner (*4*) suggested that increased platelet adhesion and thrombus formation on subendothelium exposed to whole blood could be entirely accounted for by the increased rate of platelet transport to the surface rather than enhanced platelet-surface reactivity.

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The numerous intercellular collisions in whole blood greatly increase the platelet movement perpendicular to flow streamlines; this translational movement has been described as diffusion characterized by an effective platelet diffusivity. Direct measurements indicate that platelet diffusivity increases several hundred times when red cells are added, and thus support the concept of a transport-enhancing capability for red cells (*5*).

Recently, we investigated more extensively the dependence of platelet adhesion and thrombus formation on flow conditions and analyzed the experimental results by using classical mass transport theory modified to account for a platelet diffusivity that is dependent on red cell concentration and blood shear rate (*6*). At normal hematocrit values (around 40 percent), two limiting conditions were identified: (i) a diffusion-controlled regime, below blood shear rates of 650 sec^{-1}, in which platelet adhesion is controlled predominantly by physical factors and (ii) a reaction-controlled regime, above blood shear rates of 1000 sec^{-1}, in which platelet adhesion depends primarily on alterations in platelet-surface reactivity.

In the present study, we investigated the influence of red cell concentration on platelet adhesion and thrombus formation on subendothelium exposed to blood flowing at shear rates from 50 to 10,000 sec^{-1} in order to separate diffusional effects from those due to platelet-surface reactivity.

At blood shear rates of 50 and 200 sec^{-1} (Fig. 1, a and b), platelet adhesion increases as the hematocrit value is increased from 10 to 40 percent but is independent of hematocrit values above 40 percent. At these shear conditions, virtually no thrombi form regardless of the hematocrit value. Goldsmith and Karino (*7*) observed microscopically that the radial displacement of red cell tracers reached a maximum in flowing suspensions containing 30 to 45 percent red cell ghosts (cells rendered transparent by removal of hemoglobin); at higher concentrations of ghost cells, tracer excursions became limited, although particle deformation increased. The arrival rate of platelets to the surface is probably a complex function of radial excursions and cellular deformation of red cells.

At a blood shear rate of 2600 sec^{-1}, both adhesion and thrombus formation increase continuously as hematocrit values increase from 10 to 70 percent (Fig. 1c). A similar trend is observed at shear rates of 800 and 10,000 sec^{-1} (not shown). At these high shear rates, adhesion has been shown to be relatively independent of shear rate at normal hematocrit values, a finding consistent with a reaction-limited rate of platelet-surface interaction. Thus the effect of red cells in increasing platelet interaction with the subendothelium at high shear rates and hematocrit values greater than 40 percent would appear to be based on increased red cell-surface reactivity. We have demonstrated that factors affecting platelet-surface reactivity are important at these high shear conditions. In experiments in which platelet reactivity was reduced compared to that in normal blood, either by using blood (i) depleted of von Willebrand factor (*8*, *9*), (ii) anticoagulated with increased concentrations of sodium citrate (*10*), or (iii) treated with prostacyclin (*11*), platelet adhesion rates were reduced at high shear rates but were relatively unaffected at low shear rates. However, the nature of the adhesion at high shear rates and normal hematocrit values is not entirely understood. Thrombi continue to increase as shear rate increases, although adhesion is unchanged (*6*). Thus total platelet arrival at the surface would appear to increase with shear rate; lack of increase in adhesion could be due to physical exclusion of platelets from the vessel surface or platelet incorporation by the growing thrombi. We cannot deny the possibility of an alternative physical explanation for the present results, such as increased translation of platelets from large thrombi to smaller thrombi or to the subendothelial surface due to the greater viscous forces exerted on thrombi by high concentrations of red cells.

It has been proposed that red cells play a chemical role by releasing cellular ADP upon hemolysis (*12*, *13*). The prolongation of the time required to plug a cut in a cylindrical piece of tubing through which blood was flowing in the presence of red cell-stabilizing agents

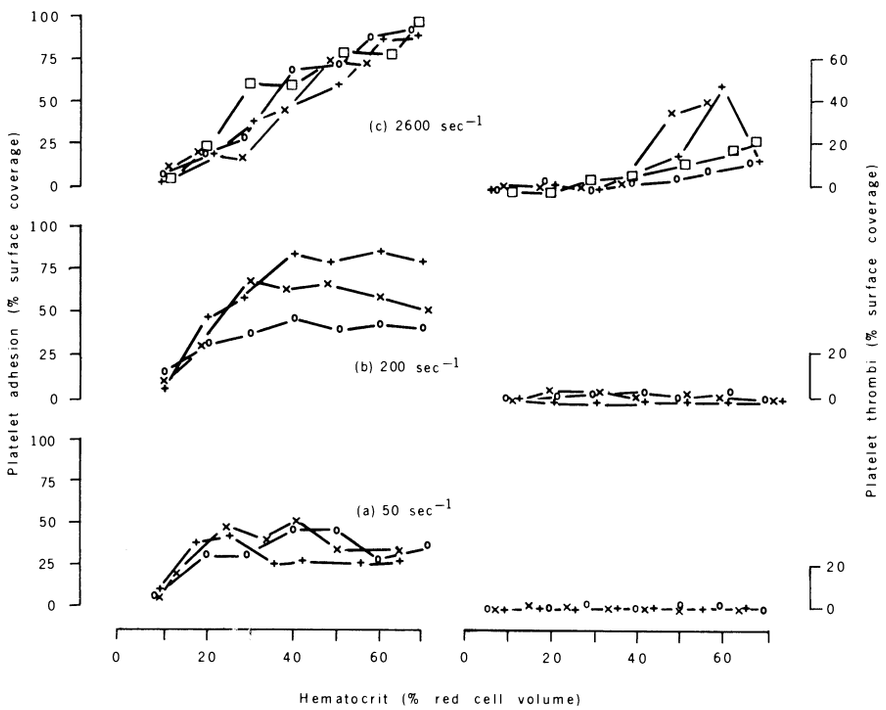

Fig. 1. Effect of red cell concentration on platelet adhesion and thrombus formation. Everted subendothelial segments (rabbit aorta), completely denuded of endothelium and mounted in an annular perfusion chamber, were exposed to human blood for a preselected average wall shear rate and time. Wall shear rates were calculated from the Newtonian velocity profile for flow in the original chamber of (a) 10 and (b) 40 ml/min for 10 minutes and, in the small chamber, of (c) 40 ml/min for 5 minutes (*9*, *17*). Blood was mixed with 305 m*M* sodium citrate (1:30 by volume), pooled, corrected to a citrate concentration of 19.7 m*M* in plasma, and centrifuged at 1500*g* for 3 minutes to obtain platelet-rich plasma and at 2400*g* for 20 minutes to obtain platelet-poor plasma and packed red cells. The platelet-rich plasma (8 ml) was combined with 18.7 ml of differing proportions of platelet-poor plasma and packed red cells to obtain reconstituted whole blood of varying hematocrit and constant platelet concentration. After exposure, platelet interaction with the subendothelium was evaluated morphometrically by light microscopy (*9*, *17*). The percentage of surface covered by platelets directly attached to the subendothelium (platelet adhesion) and the percentage of surface covered by platelet aggregates extending more than 5 μm into the lumen (platelet thrombi) were then determined. Each curve represents an experiment with a different blood sample.

was offered as proof that ADP from red cells was involved in platelet reactivity (*13*). However, these agents also directly affect platelet function; thus it is difficult to conclude that the prolonged bleeding is due to effects induced solely by red cells. Conversely, other investigators (*14*) demonstrated that the quantities of adenosine triphosphate and ADP found in blood subjected to platelet retention in glass bead columns are more consistent with amounts released by platelets (1.5:1) than red cells (10:1) and are not correlated with the amounts of hemoglobin released by red cells.

It has also been suggested that red cells deposit material on blood vessel surfaces that may affect the platelet interaction; the quantity and type of protein deposition on prosthetic surfaces have been altered by adding red cells to plasma (*15*). The nature of the protein layer is believed to affect subsequent platelet interaction with surfaces.

Although the nature of the effect of red cells at high shear rates on the formation of platelet thrombi is not entirely clear, it is apparent that red cells may play a greater role than generally suspected. Our results indicate that at low shear rates (comparable to flow in large veins) or low hematocrit values, the effect is predominantly one in which the red cells physically enhance the arrival rate of platelets (and presumably proteins) to the surface. A low arrival rate may explain why platelet thrombi form less frequently in the venous circulation and why the prolonged bleeding time in some anemias may be corrected by transfusion of red cells (*1*). At high shear rates (comparable to flow in the microvasculature) and high hematocrit values, an additional red cell mechanism results in increased thrombus formation. In polycythemia, thrombotic and bleeding episodes are a frequent complication and have been attributed in part to the reduced vascular blood flow associated with increased blood viscosity at high hematocrit values (*16*). The results of the present investigation suggest an alternative explanation for the development of platelet thrombi at high hematocrit. An extension of our results to clinical situations is that, in general, patients suffering from recurrent thrombotic episodes of a platelet origin may benefit from a reduced hematocrit and that, conversely, addition of red cells may be prophylactic in those with hemostatic defects.

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Symbolic Communication Between Two Pigeons

(Columba livia domestica)

Abstract. *Through the use of learned symbols, a pigeon accurately communicated information about hidden colors to another pigeon. Each verbal exchange was initiated with a spontaneous request for information. The two pigeons engaged in a sustained and natural conversation without human intervention.*

In a recent report, Savage-Rumbaugh *et al.* (*1*) described the first successful demonstration of symbolic communication between two nonhuman primates. They showed that chimpanzees' nonverbal communication ability could be enhanced through learning. Specifically, the chimpanzees exchanged information about food through the use of geometric symbols. They were first taught to name a number of foods by pressing buttons on which corresponding symbols were marked. Then they were taught to request hidden food by using its symbolic name. Finally, in a test of how well information about a given food could be transmitted from one chimpanzee to the other, one chimpanzee watched while some food was hidden and, in the presence of the second chimpanzee, was asked by the experimenter to indicate the symbolic name for that food. If the second chimpanzee then correctly asked for that food by using its symbolic name, both subjects were rewarded with the food. Also briefly described was a situation in which the chimpanzees spontaneously used symbols to request food from each other. Evidently, communication through the use of symbols is not an activity that is necessarily unique to man. The question naturally arises as to whether it is unique to primates.

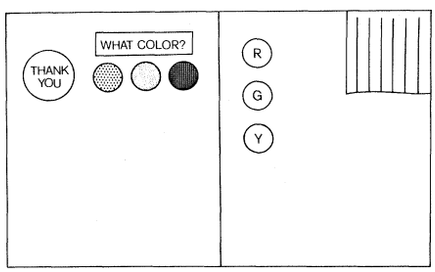

Fig. 1. Adjoining keyboards for the two pigeons. Jack's is on the left and Jill's is on the right. Jack needs information about the color recessed 5 cm behind the curtain in the upper right-hand corner of Jill's keyboard. The *R*, *G*, and *Y* on Jill's keyboard are black on white. The three keys below the WHAT COLOR? key on Jack's keyboard are yellow, red, and green from left to right.

This report presents, to our knowledge, the first instance of such symbolic communication between nonprimates—two White Carneaux pigeons (*Columba livia domestica*). Pigeons are known to communicate under natural conditions by using coos, short grunts, and wing claps (*2*). We present here data showing that their natural inclination to communicate can be enhanced through learning and, in particular, that they are able to transmit information to one another by using symbols.

The communication system was similar to that of Savage-Rumbaugh *et al.* (*1*). The pigeons expressed words or short

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