tron microscope, showed the features already described, with interdigitations between erythrocytes and lymphoid cells[2]. Rosetting lymphoid cells were in no instance labelled with ferritin. Only 4% of non-rosetting lymphocytes showed scarce ferritin granules at their surface. All erythrocytes were heavily labelled with ferritin granules, gathered in clusters arranged at fairly regular intervals. In all rosettes observed, clusters of ferritin were seen at the points of contact between erythrocytes and lymphoid cells. Neither erythrocytes nor lymphoid cells were labelled with ferritin in the control experiments.

Discussion. Our immuno-electromicroscopic study confirms, on morphological grounds, that in EA rosettes sensitized erythrocytes and lymphoid cells are held together by the link between the Fc fragment of IgG, present on the surface of the former, and specific receptors on the surface of the latter. Furthermore, this study seems to indicate that in human EA rosettes, the rosetting lymphoid cells lack surface IgG which, if present, would be labelled with ferritin. Further immuno-electronmicroscopic studies are now in progress in order to elucidate other aspects of cell-mediated immunity in vitro.

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Adhesion and aggregation of human platelets to rabbit subendothelium. A new approach for investigation: Specific antibodies

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Summary. An IgG antibody occurring in a recently transfused thrombasthenic patient inhibited all the ADP-mediated aggregations and platelet-platelet interaction (thrombus formation) on rabbit aorta subendothelium; another IgG antibody occurring in a multitransfused Bernard-Soulier patient inhibited ristocetin and bovine factor VIII mediated aggregation and platelet-subendothelium interaction.

The interaction of platelets with rabbit subendothelium in thrombasthenia and in the Bernard-Soulier syndrome has already been studied[1,2]. Whereas the thrombus formation was normal, impaired adhesion was found in the Bernard Soulier syndrome, and it was considered that an abnormal interaction between von Willebrand factor and the platelet membrane could be responsible for this abnormal platelet adhesion[2]. In contrast, adhesion was either normal or only slightly decreased in thrombasthenia with a subendothelial surface covered only by a monolayer of platelets; the most striking defect on this last disease was the absence of thrombi[1,3]. The defect associated with both those disorders of platelet function has been reported to be abnormalities in the platelet surface glycoproteins[4–6].

Recently we have had the opportunity of studying 2 different antiplatelet antibodies. The first one occurred in a polytransfused thrombasthenic patient (L...) and inhibited in vitro the aggregation of normal platelets induced by ADP[7]. The second antibody occurred in a polytransfused Bernard Soulier patient (P...) and inhibited in vitro the aggregation of normal platelets by ristocetin without any effect on the ADP mediated aggregations[8]. The aim of this study was to correlate the effect of both those platelet antibodies on platelet functions tested in the aggregometer and on the interaction of platelets with subendothelial surface of rabbit aorta. For the determination of this interaction, we have used the morphometric technique described by Baumgartner[9]. Citrated whole blood from normal subjects was circulated in the presence or absence of serum from patient L... and of purified IgG from patient P... The control whole blood was compatible in the A, B, 0, Rhesus, system with

Table 1. Effect of the L... serum on ADP-induced aggregation and interaction with rabbit subendothelium of normal human platelets. Results are expressed in percent

Final serum dilution	ADP-induced aggregation	Interaction with rabbit subendothelium	
		Adhesion	Thrombi
0	100	46	13.75
1:40	70	53	0.30
1:20	30	34	0

Table 2. Effect of the P... IgG on ristocetin induced aggregation and interaction with rabbit subendothelium of normal human platelets. Results are expressed in percent

Final IgG dilution	Ristocetin-induced aggregation	Interaction with rabbit subendothelium	
		Adhesion	Thrombi
0	100	46	13.75
1:80	50	48	9
1:40	0	24	0

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Table 3. Effect of the P... IgG on the interaction of normal platelets and on the adhesion of thrombasthenic platelets to rabbit subendothelium. Results are expressed in percent of the surface of the rabbit subendothelium covered by platelets

	Normal		Thrombasthenia Group I		Group II	
	Adhesion	Thrombi	Adhesion	Thrombi	Adhesion	Thrombi
Saline	46	13.75	67.6	0	77	0
P... IgG 1/80	48	9	52.1	0		
P... IgG 1/40	24	0	13.1	0	29.8	0

the 2 patients. Interaction was interpreted as adhesion (platelets apparently adherent to the subendothelium and spread out on it) and platelet thrombus (mass of platelets of 5 μm or more in height). Results are expressed in per cent of the total surface covered.

Results. As shown in table 1, at a subagglutinating dilution of 1:20, which in the aggregometer caused inhibition by 70% of normal platelet aggregation induced by ADP, L... serum totally inhibited thrombus formation whereas the adhesion was only decreased by 30% (34% of the surface covered by adhering platelets instead of 46% for the control). A similar inhibition in the thrombus formation was observed with a dilution (1:40) which in the aggregometer reduced only by 30% the platelet ADP induced aggregation. Figure 1 shows that the behaviour of normal blood in presence of L... serum (figure 1d) was the same as thrombasthenic blood (figure 1b), the subendothelial surface was covered with a monolayer of platelets. But at a dilution 1:40 some occasional thrombi reappeared (figure 1c) which were, however, smaller and much less frequent than observed for normal blood (figure 1a). Table 2 shows that P... IgG from a Bernard-Soulier patient at a dilution of 1:40, which completely inhibited ristocetin-induced aggregation of control platelets in PRP, caused 50% inhibition of adhesion to subendothelium. Furthermore thrombus formation was completely inhibited. At a dilution of 1:80, the P... IgG caused slight decrease in thrombus formation, adhesion was normal and the inhibition of aggregation by ristocetin was inhibited around 50%. The effect of the P... IgG may be estimated from figure 2. At a dilution of 1:40 (figure 2c) it caused similar pictures to those observed with platelets from patients with the Bernard Soulier syndrome (figure 2a, b) with the exception that the volume of Bernard Soulier platelets is increased compared to normal platelets[6]. At the dilution of 1:40, which inhibited 50% of the adhesion of normal platelets in the presence of P... IgG, 80% inhibition of adhesion was observed when using thrombasthenic whole blood (figure 2d), in which platelet adhesion can be clearly separated from platelet aggregation[2,3].

The use of serum containing an allo antibody occurring in a transfused thrombasthenic patient allows us to compare its inhibition on the ADP-induced aggregation and its effect on the platelet interaction to subendothelium. This serum reacts with a component 120,000 ± 5000 mol.

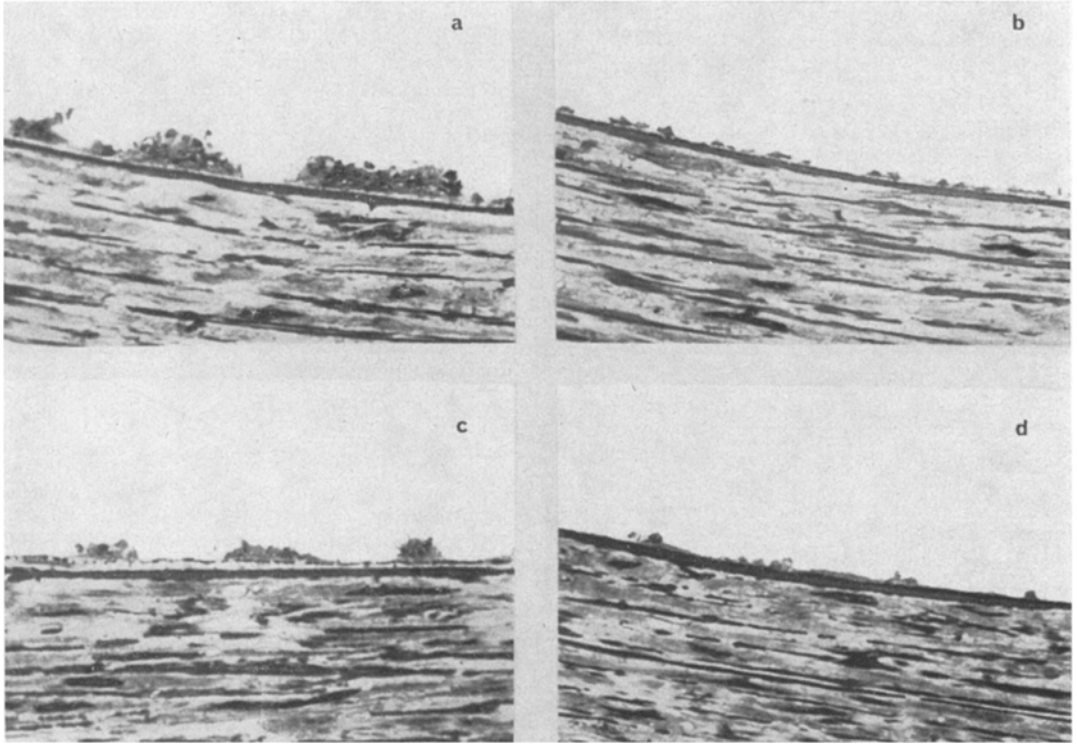

Fig. 1. Light micrographs of arterial subendothelium exposed to blood. *a*) Blood of control subject. *b*) Blood of thrombasthenic patient. *c*) Blood of control in presence of L... serum 1:40. *d*) Blood of control in presence of L... serum 1:20.

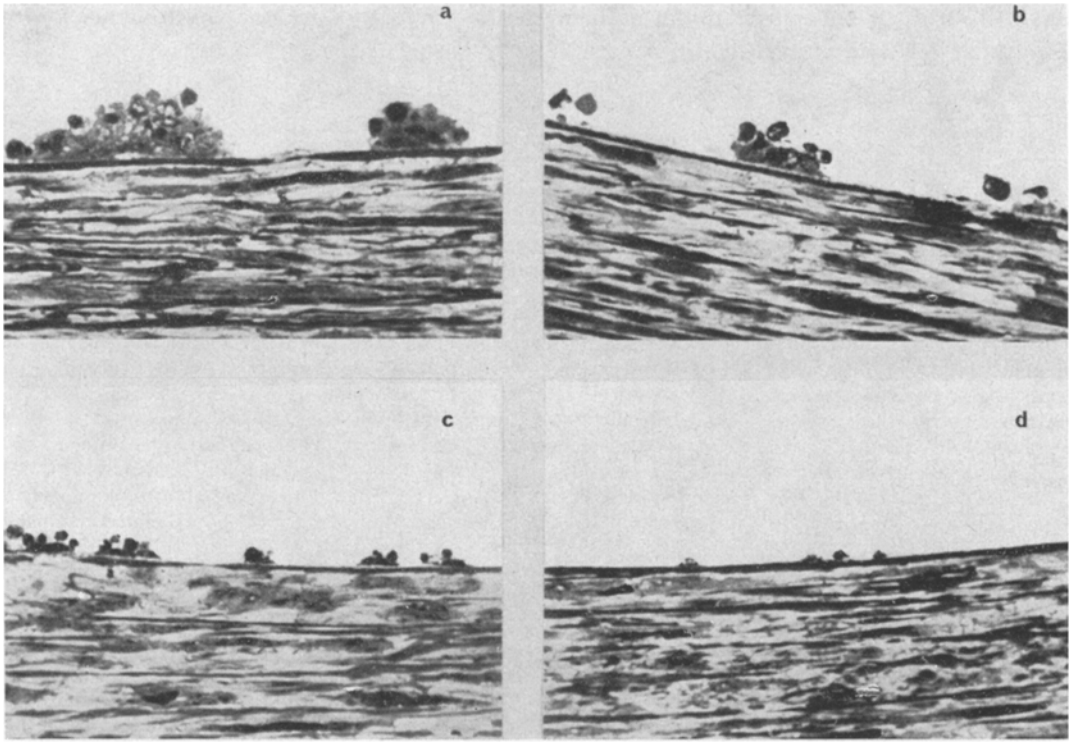

Fig. 2. Light micrographs of arterial subendothelium exposed to blood. *a*) and *b*) Blood of Bernard-Soulier patients. *c*) Blood of control in presence of P... IgG 1:40. *d*) Blood of thrombasthenic patient in presence of P... IgG 1:40.

wt present on normal platelets, absent or reduced in thrombasthenic platelets[7,10]. This component may be the glycoprotein II reduced or abnormal in thrombasthenic platelets[4,5,11,12].

Results obtained show that this serum inhibited consistently the thrombus formation in parallel with its inhibition of the ADP-induced aggregation. In contrast the percentage of the surface aorta covered by adhering platelets was only slightly decreased, but just a platelet monolayer was seen. Thus L... antibody inhibited platelet-platelet interaction more than platelet subendothelium interaction, and was able to give to normal platelets a thrombasthenic-like reactivity.

It was only recently proved that the long bleeding time in Bernard-Soulier syndrome may be related with an abnormal platelet-subendothelium interaction[1,6,12], and it was tentatively postulated that the glycoprotein I rich in sialic acid, reduced or abnormal in Bernard Soulier platelets may be related with this abnormal interaction, as well with the abnormal ristocetin and bovine factor VIII-induced aggregation[5,6,12,13]. The P... antiplatelet antibody occurring after multiple transfusions in a Bernard Soulier patient led us to compare its effect on ristocetin-induced aggregation and the platelet interaction to subendothelium. At a dilution 1:40, which inhibited completely ristocetin induced platelet aggregation, platelet adhesion to subendothelium was reduced by 50% and thrombi more than 5 μm were absent; but the adhering platelets were not a platelet monolayer and aggregates could be seen on the surface of the subendothelium. When using P... IgG with thrombasthenic platelets (table 3) which never aggregate, platelet adhesion to subendothelium was markedly decreased in the 2 groups of thrombasthenia[14]. Platelet adhesion is probably the first step of thrombus formation, the decrease of platelet adhesion observed using the P... IgG with normal platelets may modify the kinetic of the following stages and could be responsible for a delayed thrombus formation. Therefore the most striking qualitative effect of the P... anti platelet antibody seemed to be a decrease of the platelet subendothelium interaction. This P... antibody could be directed against an antigen present on normal platelets but absent or abnormal on Bernard-Soulier platelets. Speculation about similarities between this antigen and the glycoprotein I, which is abnormal in Bernard Soulier platelets[5,6,12] may be made. The inhibition by P... IgG of the adhesion of the monolayer of platelets obtained with thrombasthenic blood might suggest that the mechanisms of adhesion of thrombasthenic platelets of both groups[14] may be the same as control platelets. Besides it has been shown previously[4] that glycoprotein I was present in thrombasthenic platelets. In spite of the apparently abnormal glycoprotein surface structure of thrombasthenic platelets[11], the antigen, recognized by P... IgG, seems to remain accessible on the thrombasthenic platelet surface.

The use of such specific antibodies will provide some light on the specific role for platelet surface sites in the mechanism of cell adhesion and possibly aggregation.

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