fraction ^{125}I-18 (48% for the unabsorbed and 35% for the absorbed antiserum). That for anti-PM was high with fractions ^{125}I-15 and ^{125}I-16 (21%) and fraction ^{125}I-19 (23%), and lower (15%) with fraction ^{125}I-18.

The binding capacity for anti-CEA was 9% with fractions ^{125}I-13 and ^{125}I-14 and nil with fractions ^{125}I-18 (figure 3b). No binding capacity was detected when unabsorbed and absorbed anti-PMK and anti-PM were assayed with ^{125}I-CEA.

Conclusion. In the double diffusion test, peak 5 obtained by chromatography of MK on DEAE-cellulose reacts with absorbed anti-PMK midway between the 2 wells. The failure of peak 5 to produce precipitation lines with anti-CEA and, after further purification, with absorbed anti-PMK can be attributed to the poorer sensitivity of this method compared to radioimmunology.

The results of the double diffusion test thus indicate that the precipitation line between peak 5 and absorbed anti-PMK is due to antigen or antigens associated with breast tumors that do not crossreact with anti-CEA in this method, in spite of the considerable crossreactivity observed in the R.I.A.

Fraction 18 obtained by gel filtration of peak 5 of MK was chosen because of its high response to anti-CEA in the radioimmunological study. Fraction 18 was labeled and by gel filtration yielded fractions (^{125}I-13 and ^{125}I-14) that crossreacted with anti-CEA and also reacted with anti-PMK.

Antigen(s) in these fractions do not, however, appear to be CEA, since anti-PMK, which binds ^{125}I-13 and ^{125}I-14 to a fair extent (13% binding capacity), does not bind ^{125}I-CEA, a very pure material. On the other hand, fraction ^{125}I-18 reacts only with anti-PMK. It is therefore probable that fractions ^{125}I-13 and ^{125}I-14 contain antigens associated with breast carcinoma that crossreact with anti-CEA. It can instead be postulated that fraction ^{125}I-18 contains antigens associated with breast carcinoma that do not crossreact with anti-CEA and react only with an antibody produced using antigens extracted from this type of tumor.

Immuno-electronmicroscopic study of human EA rosettes

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Summary. The immuno-electronmicroscopic study of human EA rosettes, using ferritin-labelled anti-human IgG antiserum, showed clusters of ferritin granules at the points of contact between erythrocytes and lymphoid cells, indicating that the links between Fc fragment of IgG on the surface of erythrocytes and specific receptor on the surface of lymphoid cell correspond to the sites of morphological interaction between the 2 cell types.

In previous studies, we described the ultrastructural appearance of the interaction between rosetting lymphoid cells and erythrocytes in different types of rosettes. The highest degree of interaction, consisting of interdigitations between the 2 cell types, was observed in EA rosettes[2].

In this article, we report the results of an immuno-electronmicroscopic study of human EA rosettes using ferritin-labelled anti-human IgG antibody.

Materials and methods. Group 0 Rh positive human normal erythrocytes sensitised with a human anti-D antiserum and peripheral blood normal human lymphocytes were used for the preparation of human EA rosettes according to techniques already described for EA ox rosettes[2]. After preparation of rosettes, the cell mixture was incubated for 15 min at room temperature with a ferritin-labelled anti-human IgG antibody, washed 3 times with buffered saline and then processed for electronmicroscopy. For control, the same erythrocytes, either untreated or sensitised with the anti-D antiserum, were incubated with ferritin alone or with ferritin-labelled anti-mouse IgG antibody.

Results. Rosetting lymphocytes, in several experiments, averaged 12%. EA rosettes, observed in the elec-

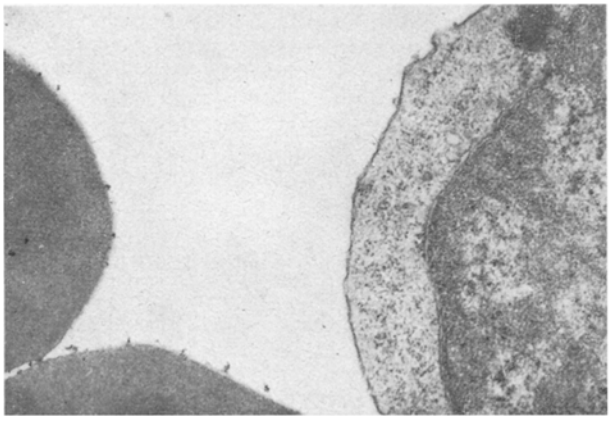

Fig. 1. Parts of 2 erythrocytes and of a lymphoid cell which are not part of a EA rosette. Clusters of ferritin granules, arranged at fairly regular intervals, are present at the erythrocytes surface. No ferritin granules are visible on the surface of the lymphoid cell. × 29000.

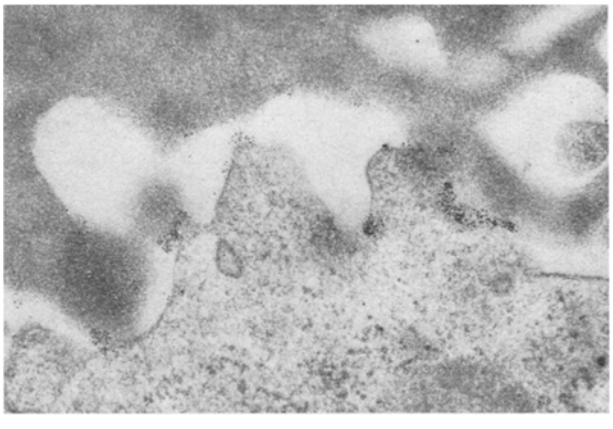

Fig. 2. Part of EA rosette. Interdigitations between an erythrocyte and a lymphoid cell are visible. The points of contact between the 2 cell types are heavily labelled with ferritin. × 46000.

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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