The non-reducible changes brought about by copper phenanthroline are very specific; no other microsomal proteins seem to be involved except those induced by β-naphthoflavone. The reasons for this specificity remain to be ascertained; it may be an intrinsic chemical property of the proteins represented by bands D and E or it may arise from the specific nature of their molecular environment. In either case, the fact that the synthesis of these proteins had been recently induced may have a bearing on the specificity of the effect.

We are grateful to the Science Research Council (GR/A/41007) for financial support. P. R. M. thanks Dr. M. J. Coon and Mrs. S. Dahl for helpful discussions.


The Release of Erythrocyte Membrane Antigens to the Plasma as Membrane Microvesicles during the Storage of Human Blood for Transfusion

WENDY F. COLE and MARTIN G. RUMSBY

Department of Biology, University of York, Heslington, York YO1 5DD, U.K.

and GEORGE H. LONGSTER and L. A. DERRICK TOVEY
Regional Transfusion Centre, Bridle Path, Leeds LS15 7TW, U.K.

During the storage of human blood for transfusion at 4°C in ACD plasma (citric acid/sodium citrate/dextrose) there is an observed decrease in erythrocyte agglutinability (Rosenfield et al., 1971; Longster et al., 1978). This decrease was observed for several erythrocyte antigenic groups. The progressive appearance of erythrocyte antigenic activity in plasma from donations stored for transfusion has also been demonstrated by specific inhibition studies (Longster et al., 1978). The increase in specific inhibition in the plasma corresponded well with the observed decrease in erythrocyte agglutinability. This communication explores the process by which erythrocyte surface antigenic groups are lost to the plasma during storage at 4°C in vitro and whether or not specific antigenic groups are lost preferentially from the erythrocyte membrane.

It is known that lipid is lost from the erythrocyte membrane during the storage in vitro of human blood for transfusion (Haradin et al., 1969; Rumsby et al., 1977). We now know that the bulk of this lipid is recoverable from the plasma as membrane-bounded microvesicles which contain haemoglobin (Rumsby et al., 1977). Preliminary analysis of the membrane proteins of these microvesicles by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis shows that the normal spectrin polypeptide bands of the erythrocyte membrane are markedly depleted and that glycoproteins are present together with other protein components (Rumsby et al., 1977). It therefore seemed possible that the observed increase in erythrocyte antigenic activity in the plasma of stored donations may arise from the release of erythrocyte surface antigens in microvesicles rather than that such antigens are lost independently of membrane by, for example, the action of proteolytic enzymes. The present studies were designed to investigate this point.

Several donations of human blood of various groups, e.g. ABO, MN, P etc., but all Rhesus positive, were stored under normal transfusion service conditions at 4°C in ACD plasma for up to 8 weeks. At fortnightly intervals samples of premixed blood, after testing for sterility, were centrifuged at 4°C and 1000 g for 20 min. The plasma was recovered and centrifuged again at 4°C and 2000 g for 10 min to ensure the removal of leucocytes and platelets. Duplicate samples of the 2000 g plasma were
Plasma samples are prepared from human blood donations stored in ACD plasma at 4°C for up to 8 weeks. Experimental details are given in the text. Agglutination score is the score obtained with antiserum after the addition of stored plasma with control cells expressed as a fraction of the score obtained with antiserum after addition of saline with the same control cells. The results shown are the means from four separate donations.

Then centrifuged at 4°C and 100000g for 60 min. One supernatant from this procedure was further centrifuged at 150000g for 60 min. From week 2 to week 8 increasing amounts of the red microvesicle pellet were recovered by centrifuging the 2000g plasma at 100000g, in agreement with previous findings (Rumsby et al., 1977). Further centrifugation of the 100000g plasma at 150000g only sedimented traces of a microvesicle pellet. No microvesicles were obtained with plasma from freshly taken donations of blood.

The different plasma samples were tested for specific inhibition with various human antisera. Doubling dilution titrations of antiserum were set up and an equal volume of plasma was added to each with mixing. After 10 min at room temperature (22°C), during which time any inhibition should occur, an equal volume of a 2–5% suspension of erythrocytes of the appropriate group in 0.15M-NaCl was added and the tests were mixed and incubated at 20°C for 2 h. The score obtained after the addition of plasma to each antiserum was expressed as a fraction of the score obtained after the addition of 0.9% NaCl with the same control cells. Scoring was as described by Stratton & Renton (1958).

Changes occurring in the inhibitory properties of the supernatant plasmas over the 8 weeks of storage for human anti-A serum and A, cells are shown in Fig. 1. The 2000g plasma caused increasing inhibition up to 8 weeks of storage but, after sedimentation of membrane microvesicles from this plasma at 100000g, the inhibitory properties were markedly decreased. Centrifugation of 100000g plasma at 150000g effectively removed the remaining inhibition. Similar trends of results were obtained with anti-A1, anti-H, anti-M and anti-P1 human sera with appropriate cell types. The degree of inhibition

Fig. 1. Changes occurring in the inhibitory properties of 2000g (○), 100000g (●) and 150000g (□) supernatant plasmas with human anti-A serum and A1 cells.
Saline-washed packed erythrocytes from human blood donations (group A1, Rhesus positive) were resuspended in group O adsorbed serum at their original haematocrit. Storage for 8 weeks and plasma preparation are as described in the text. Agglutination score is as described for Fig. 1 and in the text.

observed with anti-M serum was less pronounced and was only apparent after longer periods of storage. The slight inhibition detected in the 150000g plasma, especially towards the end of the storage period (Fig. 1), may be due to incompletely sedimented fragments of membrane which carry erythrocyte antigenic activity or to traces of surface antigenic groups which are released to the plasma free of membrane during storage at 4°C. The apparent slight inhibition detected with plasma from freshly taken donations may arise from the subjectivity of the scoring procedure, but is, more likely, due to the presence of trace amounts of soluble blood-group substances in human plasma. To test this latter idea, donations of blood (group A1, Rhesus positive) were taken as above. Erythrocytes were sedimented and washed twice with 0.15M-NaCl. Erythrocytes were then resuspended at their original haematocrit in O serum, which had been adsorbed for anti-A and anti-B sera, thereby providing an environment free of blood-group reactive substances. These donations were treated similarly to those above, and inhibition results are shown in Fig. 2 for plasma centrifuged at 2000g and 100000g only. The absence of inhibition at week 0 confirms the suggestion that the slight inhibition detected in freshly taken samples (Fig. 1) arises from secreted blood-group-active substances already in the plasma. Subsequently, the trend of inhibition observed with increasing storage for plasma in Fig. 2 is similar to the findings of Fig. 1.

The findings in this communication indicate clearly that erythrocyte surface antigenic activity lost to the plasma during the storage of human blood for transfusion is almost exclusively associated with the membrane-bound microvesicles. The most likely explanation for this is that the erythrocyte antigens are released as integral components of the membrane of the microvesicles. We now want to determine whether the density

---

Fig. 2. Changes occurring in the inhibitory properties of 2000g (○) and 100000g (●) supernatant plasmas devoid of secreted blood-group substances using human anti-A serum and A₁ cells
and character of these antigenic groups released on the microvesicle membrane is similar to that of the parent erythrocyte membrane.

This work is supported by funds from the Science Research Council, with a research studentship CASE award to W. F. C., and the Department of Health and Social Security.

Longster, G. H., Tovey, L. A. D., Barnes, Y. & Rumsby, M. G. (1978) Vox Sang. 34, 193–199

Phospholipases in the Membranes of Rat Kidney Lysosomes

SUSAN F. SEAGER, MITCHELL FRY and DAVID T. PLUMMER

Department of Biochemistry, Chelsea College, London SW3 6LX, U.K.

To carry out their normal cellular functions, lysosomes must have the ability to fuse with other membranes. The mechanism of membrane fusion is still not fully understood but one suggestion is that local conversion of phospholipids into lysophospholipids at fusion sites leads to micellization and bilayer instability with the consequent mixing of membrane components (Lucy, 1970). The stable bilayer then re-forms as the lysophospholipids diffuse away from the fusion site and are subsequently reacylated. Phospholipases could therefore be important in membrane fusion. Furthermore, some drugs that stabilize kidney lysosomes also seem to inhibit a membrane-bound phospholipase (Fry & Plummer, 1978). In view of these reports, the lipolytic activity of lysosomal membranes was investigated.

Fig. 1. The variation of acid phosphatase activity in a kidney homogenate with sucrose concentration

A 10% (w/v) homogenate of rat kidney was prepared in a range of sucrose concentrations (0–0.45M) buffered with 10mm-triethanolamine/butyrate, pH 7.5, then centrifuged at 10000g for 10min. The acid phosphatase activities in the particulate fraction (●) and the supernatant (○) were then determined.