Ethanolamine inhibits the fusion of erythrocytes by (polyethylene glycol) 6000 and by cell swelling.

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In previous work it was shown that, when monolayers of human erythrocytes were induced to swell in the presence of 10 mM Ca$^{2+}$ by the entry of small molecules, e.g. malonamide or poly(ethylene glycol) 400 (PEG 400), many of the swelling cells fused before they lysed. By contrast in the absence of Ca$^{2+}$, lysis occurred without any cell fusion [1].

We have now observed that this fusion process is preceded by hemi-fusion (i.e. membrane fusion without cytoplasmic fusion) as demonstrated by the rapid movement of a fluorescent membrane probe (carbocyanine) from labelled to unlabelled cells. Unlike fusion, hemi-fusion occurs both in the presence and absence of added Ca$^{2+}$, but it is inhibited by the addition of 10 mM EGTA.

Hemi-fusion, and hence complete fusion was strikingly inhibited by pre-incubation of the cell monolayers with 20 mM ethanolamine at pH 8.5 but not at pH 7.4. Pre-incubation with other amino alcohols (e.g. 6-amino I-hexanol) similarly inhibited hemi-fusion provided they were also included in the malonamide solution. Glycerol, ethylene glycol and urea were not inhibitory. A membrane-impermeable molecule (e.g. sucrose) was required during the pre-incubation to protect the cells against lysis. Hemi-fusion was also inhibited by high concentrations of choline. Unlike other inhibitors, choline was effective at pH 7.4 but not pH 8.5. Equally, in the absence of sucrose, choline lysed the cells only at the lower pH. These observations indicate that inhibitors of hemi-fusion act inside the erythrocytes.

Human erythrocytes visibly became spherical during the pre-incubation of the cell monolayers with ethanolamine. Upon its removal, this shape change was reversed. By means of a fluorescence-activated cell sorter, the shape change was also observed with erythrocytes in suspension (Fig. 1).

In addition to becoming more spherical, erythrocytes in suspension that had been pre-treated with ethanolamine were also more resistant to lysis resulting from the entry of malonamide. This is apparent from the light scattering profiles of the cells (Fig. 1).

Other amino alcohols that inhibited hemi-fusion in swelling erythrocytes induced a similar change in cell shape. When monolayers were prepared from cells which had already been pre-treated with ethanolamine in suspension, hemi-fusion induced by the entry of malonamide was still inhibited. This indicates that the inhibition of hemi-fusion in the original protocol was not due to a loss of contact between the cells in the monolayer when they became spherical. No evidence was obtained for any effect of ethanolamine on endogenous enzymes such as phospholipase A$_2$, phospholipase C, phospholipase D, proteinases, or phosphatases.

When monolayers of human erythrocytes were exposed in previous work to Ca$^{2+}$ in the presence of the calcium ionophore A23187, phosphatidylerine (normally only present in the inner leaflet of the plasma membrane) appeared at the cell surface, and this facilitated the fusion of erythrocytes induced by cell swelling in the presence of Ca$^{2+}$ [2].

It is possible that an interaction (mediated by electrostatic and hydrogen bonds) between ethanolamine in the cytoplasm and phosphatidylerine in the inner leaflet causes the cells to adopt the observed spherical shape. This interaction might conceivably also prevent phosphatidylerine from moving to the outer leaflet of the erythrocyte plasma membrane during fusion protocols involving cell swelling. However, the possibility that ethanolamine may interfere with hemi-fusion by associating with phosphatidylerine after it has moved to the outer leaflet cannot be excluded at present. Inhibition by ethanolamine of the prothrombinase assay [3], which we employed to determine surface exposure of phosphatidylerine in erythrocytes, has precluded the assay from being used to distinguish between these two alternatives in the present experiments.

It is relevant to our interpretation that, from an NMR study of the interactions of phloretin and bilayers of phosphatidylcholine, it has been concluded that hydrogen-bond formation between the phloretin hydroxyl group and phospholipid phosphate is involved in altering the effective dipole moment at the membrane surface by phloretin [4].

It has previously been shown that monolayers of human erythrocytes exhibit hemi-fusion in 40% poly(ethylene glycol) 6000 (PEG 6000) [5]. Erythrocytes are permeabilised by the dehydrating polymer solution [6] and, when it is replaced by an isotonic buffer, they swell beyond their original volume. Some of the hemi-fused cells then fuse completely before lysing [5]. We now report that, although pre-incubation of erythrocytes with 20 mM ethanolamine prior to their exposure to PEG 6000 does not prevent hemi-fusion, the cells fail to fuse on rehydration at pH 8.5. They also lyse far more slowly than control cells (treated with ethylene glycol instead of ethanolamine). The mechanisms of these effects of ethanolamine on cells treated with PEG 6000 are being investigated.

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![Fig. 1. Light scattering by human erythrocytes.](image-url)