the membrane lesion permitted the escape of the molecules.

The tentative conclusions drawn from the above observations are supported by the immunological approach of C. Howe, who has shown that antibodies prepared against the single-torus protein do not agglutinate intact erythrocyte (personal communication). Also, Hoozeveen, Juliano, Coleman & Rothstein (1970), using intact human erythrocytes and erythrocyte 'ghosts' that had been labelled with a fluorescent stilbene compound, have shown that on extraction at low ionic strength all the fluorescence remained with the residual membrane fragments. Both these observations suggest that the proteins that are released when the intact erythrocyte 'ghost' undergoes fragmentation at low ionic strength come from the inner surface of the membrane.

The possibility remains that the proteins under discussion are predominantly adsorbed cytoplasmic contaminants. Hoozeveen *et al.* (1970) present a calculation that argues very much against this being the case. The present author considers that, as the proteins have bound to the 'ghosts' through the extensive washings during which the haemoglobin is released, it is unlikely that other cytoplasmic proteins would remain bound. Even if in the intact erythrocyte an equilibrium exists between membrane-bound and cytoplasmic protein, it is reasonable to consider the proteins to be membrane-associated, though not membrane 'structural' proteins.

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The Proteins of Erythrocyte Membranes: Where are They?

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Among the proteins present in human erythrocytes there are two major components that are located, at least in part, on the external surface of the cell. These have been located by their ability to react with a non-penetrable reactive reagent, formylmethionyl sulphone methyl phosphate, of

extremely high specific radioactivity. These two components have apparent molecular weights, determined by sodium dodecyl sulphate-gel analysis, of about 105 000 (a) and 90 000 (b). Each is present in approx. 4×10^6 copies/cell; together they might cover at least 25% of the cell surface, and probably more. Component (a) appears devoid of sialic acid and carbohydrate. Component (b) bears about 50% of the cell-surface carbohydrate and about 70% of the cell-surface sialic acid. Its apparent molecular weight of 90 000 is therefore likely to be misleading, since a large fraction of its mass (perhaps half) is carbohydrate. Components (a) and (b) are now being 'fingerprinted'.

The reagent also labels phosphatidylserine and phosphatidylethanolamine. Only slight labelling of these lipids can be detected on the external surface of erythrocytes although they are heavily labelled when 'ghosts' are exposed to the reagent. This suggests either (1) that these phospholipids are predominantly built into the inner surface of the cell or (2) that they are present equally on both inner and outer surfaces, but are less available [being possibly screened by proteins (a) and (b)] on the outer surface than on the inner surface. It is not possible, as yet, to distinguish between these two alternatives.

Separation and Partial Characterization of Some Human Erythrocyte Membrane Protein Components

By M. J. A. TANNER and D. OWENS. (*Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.*)

Extraction of human erythrocyte 'ghosts' with EDTA solutions containing 2-mercaptoethanol (Marchesi, Steers, Marchesi & Tillack, 1970) followed by solutions containing 1 M-sodium chloride results in the solubilization of many of the bands seen on polyacrylamide-gel electrophoresis of intact 'ghosts' in sodium dodecyl sulphate and 8 M-urea. The insoluble residue remaining contains 47% of the protein, 58% of the total phosphate, 57% of the cholesterol, 75% of the hexose and 50% of the sialic acid present in the intact membrane, and gives only a diffuse band over the molecular-weight range 65 000–40 000 and a low-molecular-weight 'mini-protein' band (Laico, Ruoslahti, Papermaster & Dreyer, 1970) on sodium dodecyl sulphate-gel electrophoresis. Tryptic-peptide 'maps' of erythrocyte 'ghosts' show a small number (17–20) of intensely staining peptide spots against a high background of a large number of peptides staining with low intensity. 'Ghosts' extracted as above (salt-extracted 'ghosts') yielded a tryptic-peptide