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

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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

'map' that contained a large number (13) of these intensely staining spots against a low level of background staining. Tryptic-peptide 'maps' of spectrin (Marchesi *et al.* 1970), the major protein removed from the 'ghosts', showed a very large number of peptides that were poorly resolved from each other, and also contained the small number of the intense spots that were not present in 'maps' of salt-extracted 'ghosts'.

The salt-extracted 'ghosts' were chromatographed on Sephadex G-150 in the presence of 1% sodium dodecyl sulphate. The E_{280} pattern showed a broad spread of protein with a peak of material at 65 000–25 000 molecular weight, and a peak of material in the range 12 000–6 000 molecular weight. Amino acid analysis showed differences between the fractions. Carbohydrate analysis showed that the higher-molecular-weight peak was associated with high contents of galactose (260 nmol/mg), *N*-acetylglucosamine (220 nmol/mg) and sialic acid (300 nmol/mg), this material being nearly 30% carbohydrate by weight. The low-molecular-weight peak contained large amounts of *N*-acetylgalactosamine (230 nmol/mg), galactose (270 nmol/mg) and lipid.

The two protein peaks gave simple but different tryptic-peptide 'maps'. The positions of the peptides suggested that a major component of the higher-molecular-weight peak was not present in the low-molecular-weight peak, but did not exclude the possibility that the polypeptide present in the low-molecular-weight peak was also present in the high-molecular-weight peak. 'Fingerprints' of thermolysin digests of the material of the two peaks showed that a polypeptide component was indeed present in both peaks.

Digestion of the material of the low-molecular-weight peaks by carboxypeptidases A and B in the presence of sodium dodecyl sulphate released threonine and valine in approximately equimolar amounts (1 residue/200 residues of protein), with a slightly lower yield of alanine and leucine. Kinetic experiments suggested that the order of release was valine, threonine, alanine, leucine. It was not possible to distinguish between a single polypeptide with a *C*-terminal sequence of -Leu-Ala-Thr-Val and three or four chains with differing *C*-termini present in similar molar amounts. The higher-molecular-weight peak showed a similar pattern of release of the same amino acids in high yield (threonine, 1.3 residues/200 residues), serine being the only additional amino acid released (0.33 residue/200 residues). Each of the fractions obtained from the gel-filtration column showed the release of threonine, valine, alanine and leucine. The kinetics of the release were very similar for each fraction and the magnitude of the yields followed the order threonine > valine > alanine > leucine.

Salt-extracted 'ghosts' appear to contain two major groups of polypeptides. One of these, present in the higher-molecular-weight peak, is probably identical with the sialoglycoprotein isolated by several workers (Springer, Fletcher & Pavlovskis, 1967; Blumenfeld, Gallop, Howe & Lee, 1970). In addition, a distinct low-molecular-weight component aggregates throughout a wide molecular-weight range in 1% sodium dodecyl sulphate, and may be composed of a single polypeptide chain or a very small number of polypeptide chains that aggregate in such a way that the composition of the complex is the same irrespective of its molecular weight. This component is probably similar to the membrane miniprotein described by Laico *et al.* (1970).

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Some Properties of Mitochondrial Membrane Proteins Dissolved in Chloroform-Methanol

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There is considerable experimental evidence suggesting that the major interaction between lipids and proteins in a membrane is hydrophobic, and a number of models for a membrane have been proposed on this basis (Benson, 1966; Lenard & Singer, 1966; Sjöstrand & Barajas, 1970; Vanderkooi & Green, 1970). Therefore organic solvents may provide a suitable medium for proteins that lie mainly within the hydrophobic region of a membrane. There are a few reports of organic solvents being used to dissolve membranes (Blumenfeld, 1968; Green, Haard, Lenaz & Silman, 1968; Lowden, Moscarello & Morecki, 1966; Rega, Weed, Reed, Berg & Rothstein, 1967; Schnaitman, 1969; Zahler & Wallach, 1967; Zwaal & van Deenen, 1968), but little advantage has been taken of this fact except to separate protein from phospholipid by gel filtration on