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Factors Affecting the Interactions of Proteins Isolated from Erythrocyte Membranes

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Proteins may be isolated from membranes either by a series of extractions with a sequence of reagents of increasing severity or by a one-step procedure aimed at releasing all the protein into solution by exposure of the membrane to just one solvent. Both approaches have their merits, the advantage of the latter being that it produces a solution containing all the membrane proteins without their having been exposed to a range of extractive procedures, its major disadvantage being the very complexity of the solution it produces.

The butanol procedure one of us devised some time ago (Maddy, 1966) had the merit of producing virtually all the protein of erythrocyte 'ghosts' in an aqueous solution in a relatively undamaged state, but unfortunately this protein solution proved to be extremely difficult to fractionate. However, if the butanol procedure is carried out at low ionic strength in the presence of EDTA the resulting protein solution can be readily fractionated. Using polyacrylamide-gel electrophoresis and analytical centrifugation we have demonstrated in this solution (i) a fraction identical with that part of the membrane's protein that may be extracted from the 'ghost' by EDTA solution alone (Marchesi, Steers, Marchesi & Tillack, 1970), (ii) a fraction containing most and probably all of the membrane's sialoprotein, and (iii) a component with a pronounced tendency to aggregate into an intractable complex.

Three sets of interactions have been detected as factors responsible for aggregation of the isolated membrane proteins: a hydrophobic force, identified by the effects of increasing the ionic strength of the solution, a bivalent-ion bridge effect reversed by EDTA or low pH, and an electrostatic interaction between sialoproteins and other protein of opposite charge. Other interactions not destroyed by sodium dodecyl sulphate or guanidine hydrochloride have been observed, but their physical basis is unknown. The influence of these forces on the behaviour of ox erythrocyte membrane proteins after an EDTA-butanol isolation will be considered. The relative effects of the interactions will differ in different methods of protein isolation, but whatever method is employed their existence must be recognized and accounted for.

Recombination *in vitro* of Proteins and Lipids from Mammalian Erythrocyte Membranes

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Membrane apoproteins of mammalian erythrocytes, isolated by the butanol fractionation procedure of Maddy (1966), have been used in recombination experiments carried out in aqueous media with both erythrocyte total lipids and phospholipids. By using density-gradient centrifugation it was found that no recombination occurred at physiological pH, probably owing to repulsion of both negative net charges of proteins and lipids. However, recombinations turned out to be possible with negatively charged sonicated lipids and positively charged proteins between pH 3.5 and 4.5 (Zwaal & van Deenen, 1970). For this purpose the sialic acid was removed from either the proteins or the intact erythrocytes by treatment with neuraminidase before the recombination procedure. The stoichiometry of the reaction appeared to be dependent on pH. The differences in lipid uptake as a function of pH for apoproteins were compared with the differences in uptake of additional lipids by sonicated 'ghost' suspensions and by lipoproteins obtained after pentanol solubilization of erythrocyte membranes (Zwaal & van Deenen, 1968). It appeared that between pH 3.5 and 4 the amount of lipid taken up by the apoproteins was identical with the amount taken up by the 'ghost' suspensions (or lipoproteins) plus the amount that was already present. The measurements on 'ghosts' suggest that at pH 4 no combination with excess of lipids may occur, whereas the apoprotein at this pH are still able to recombine up to a phospholipid/protein ratio comparable with that of the original membrane.

Recombinations carried out with individual phospholipids from human erythrocyte membranes confirm the notion that opposite charges of lipids and proteins are essential for the initial interaction. Additional confirmation could be derived from recombinations above pH 5.5 with negatively charged proteins and positively charged liposomes containing stearylamine. In addition, it appeared that once the recombination had been formed at pH 4 addition of sodium chloride or change in pH from 4 to 7 was ineffective in splitting the complex. On the other hand no recombination could be obtained in the presence of sodium chloride (0.2M). The extractability patterns of lipids from the