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Electrostatic interactions at the plasma membrane

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[Plate 4]

Hydrogen-ion titration has been used to detect the presence of charged groups on the human red-cell plasma membrane. The findings are discussed in terms of the effect of the local environment on electrostatic interactions between the charged groups.

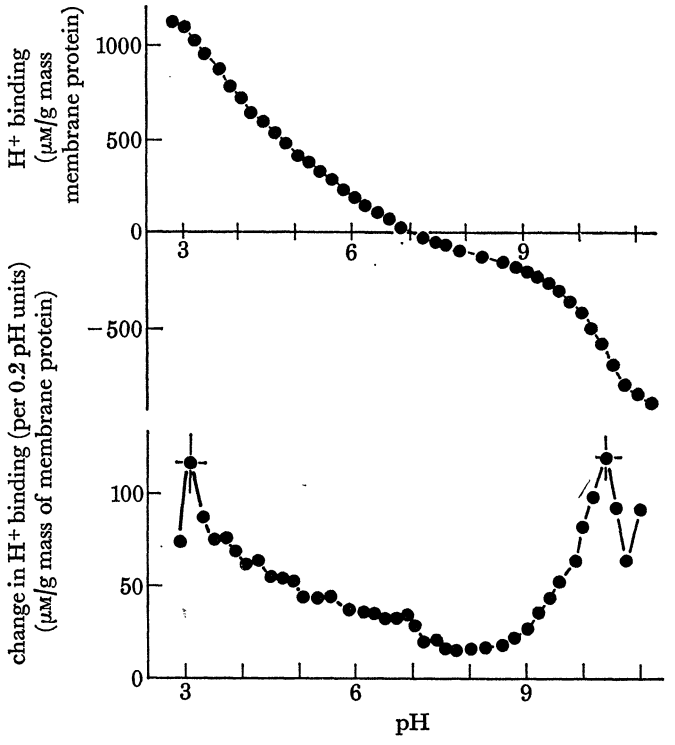
Introduction

It is probable that the plasma membrane is closely involved in the mechanisms regulating the response of the cell to its environment. In this respect, Dr Hallam and I have been particularly interested in effects of local changes in pH and ionic composition on the cell surface. As a method of study we have been titrating various plasma membranes with acid and alkali. In the past, titration curves have provided one of the simplest methods of detecting conformational changes in proteins (Tanford 1962; Steinhardt & Beyechok 1964) and have also been used to study the electrostatic disaggregation of the highly charged membranes of *Halobacterium halobium* (Brown 1965). The aim of the present study was to identify titratable charged groups on plasma membranes and, at the same time, monitor any effects of altering membrane charge on the arrangement of membrane components.

Membrane titration

Human red-cell membrane 'ghosts' prepared according to Dodge, Mitchell & Hanahan (1963) were suspended at approximately 2 mg ml^{-1} in 120 mM potassium chloride to a final volume of 4 ml at pH 7.0. Potassium hydroxide and hydrochloride acid were used as titrants and the output from a combined pH electrode was continuously monitored on a chart recorder. All titrations were performed at 32 °C under nitrogen.

The titration curve of red-cell plasma membranes (figure 1, upper graph) was obtained by subtracting the appropriate control titration (of 120 mM potassium chloride alone) and the results are expressed as moles of hydrogen ion bound (or dissociated)/g membrane protein, taking pH 7.0 as the point of reference. The presence of titratable groups on the membrane can be detected from an analysis of the titration curve by plotting pH against the differential of hydrogen ion bound (figure 1, lower graph). This latter plot more clearly indicates where association and dissociation of hydrogen ions occur. The main features of the differential titration curve are a peak at pH 10.5 ± 0.2 and a general increase in hydrogen ion binding between pH 7 and pH 3 with a maximum at pH 3.1 ± 0.2.



FIGRUE 1. Normal (upper trace) and differential (lower trace) titration curve of human red-cell plasma membranes in 120 mM potassium chloride. Each half of the titration was started from pH 7. For clarity, only the standard error of the mean of the two main peaks are shown (13 observations).

CHARGED GROUPS

It has been possible, by selective chemical and enzyme treatment, to identify many of the charged groups responsible for the shape of the titration curve. However, for the present discussion we are more interested in any effects that a change in membrane charge could have on the arrangement and organization of membrane components. The results shown in figure 1 indicate that membrane charge would be altered by proton association as the pH is lowered from pH 7.5 to pH 5.0. Electron microscope examination of freeze-fracture preparations of the membranes sampled at these two pH values show a difference in lateral organization of the membrane components (figure 2, plate 4). A higher degree of aggregation of intramembrane particles occurs at the lower pH.

Redistribution of membrane components in red cell and other cell plasma membranes has been reported to result from changes in pH and ionic strength (Pinto da Silva 1972), lectin and antibody binding (Nicolson & Singer 1973), selective enzyme digestion (Nicolson 1972), and during virus induced malignant transformation of cells (Nicolson 1971). All these changes could result from alterations to the electrostatic interactions between titratable charged groups attached to membrane components. In general, we would suggest that any disturbance to the electrostatic balance between charged groups on membrane proteins and glycoproteins would affect the lateral organization of these components in the membrane. This could result from changes in pH or ionic strength in the local environment, the presence of certain divalent cations (Hallam & Wrigglesworth 1973), the close approach of another charged surface as in cell–cell contact, and also from specific interaction of membrane charged groups with charged

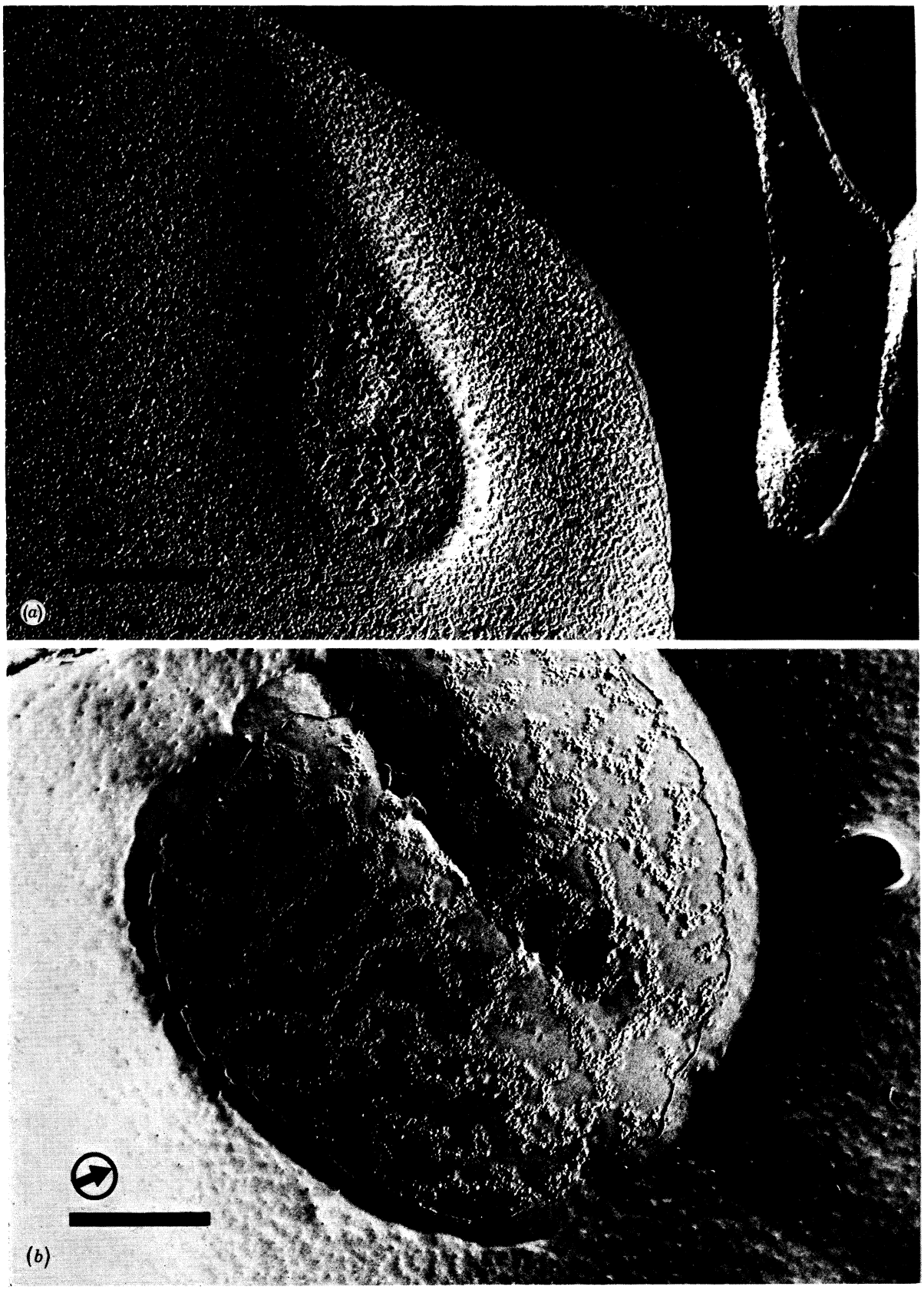


FIGURE 2. Freeze-fracture electron micrographs of red-cell plasma membranes in 10 mM potassium chloride containing 5 mM potassium phosphate + 5 mM sodium acetate at (*a*) pH 7.5 and (*b*) pH 5.0. Aggregation of intramembrane particles is intense at pH 5.0. (Bar, 0.5 μm; arrow indicates direction of shadowing.)

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molecules such as peptide hormones, toxins, lectins or antibodies. Because of a low dielectric constant, non-polar regions of the membrane offer little electrostatic barrier to charged group interactions. In this respect, the inside surface of the plasma membrane is in electrostatic 'communication' with the outer surface. Alteration or redistribution of charged groups on the outer surface could easily cause a reorganization of inner-surface charged groups and could be important in the regulatory mechanisms controlling the response of the cell to its environment.

The work reported here was done in collaboration with Dr C. Hallam.

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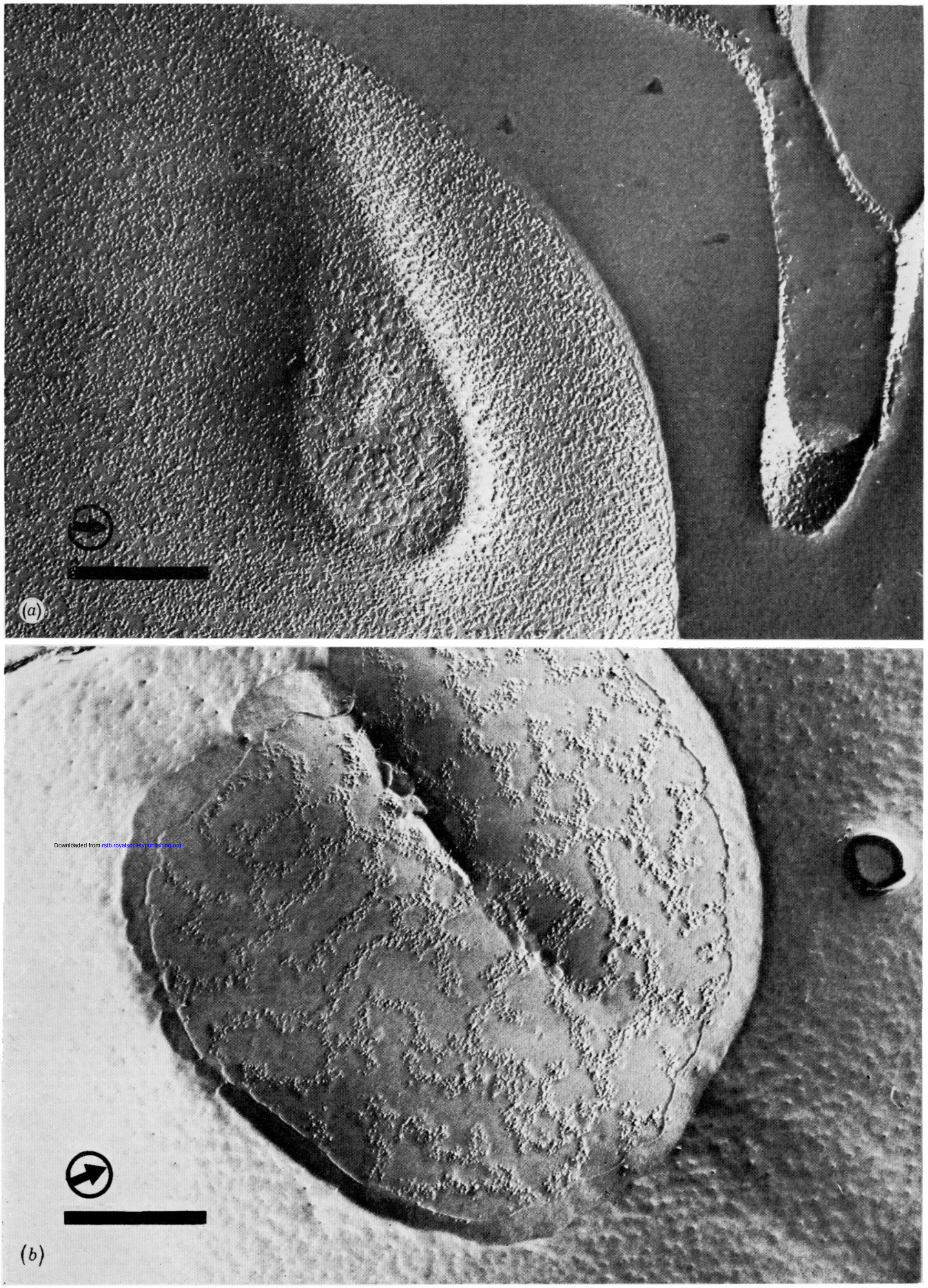

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