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Investigation of electrical charge distribution on human erythrocytes by mutual adsorption of hydrosols
by Ivan Yip-Keung Choi

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:
Rouleaux formation of human red blood cells is usually found in high molecular weight protein media when the blood flow rate is low. This formation may be due to electrostatic attraction between positively-charged sites on the surfaces of the red blood cells and the negatively-charged proteins. In order to test this assumption, acetaldehyde-fixed red blood cells were stained, while in suspension, with two electrical forms each of colloidal silver iodide and colloidal iron oxide. One was the positively charged colloid which could be mutually adsorbed on the negative sites of the surface of the red cells; the other was the negatively charged form which might be adsorbed on the positive sites on the surface of the red cells.

Transmission electron microscopy and scanning electron microscopy were the tools used to investigate the red blood cells. Results shown by electron micrographs support the hypothesis that the negative charge on the red cells is distributed over the entire surface, whereas the positive charge is not uniformly distributed but is found to be stronger on the side of the red blood cells.

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Date May 27, 1970

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ABSTRACT

Rouleaux formation of human red blood cells is usually found in high molecular weight protein media when the blood flow rate is low. This formation may be due to electrostatic attraction between positively-charged sites on the surfaces of the red blood cells and the negatively-charged proteins. In order to test this assumption, acetaldehyde-fixed red blood cells were stained, while in suspension, with two electrical forms each of colloidal silver iodide and colloidal iron oxide. One was the positively charged colloid which could be mutually adsorbed on the negative sites of the surface of the red cells; the other was the negatively charged form which might be adsorbed on the positive sites on the surface of the red cells.

Transmission electron microscopy and scanning electron microscopy were the tools used to investigate the red blood cells. Results shown by electron micrographs support the hypothesis that the negative charge on the red cells is distributed over the entire surface, whereas the positive charge is not uniformly distributed but is found to be stronger on the side of the red blood cells.

INTRODUCTION

A human red blood cell, or erythrocyte, is a cell consisting of a plasma membrane and containing a hemoglobin solution with none of the complicating features associated with the presence of mitrochondria and other subcellular components. Its undisturbed shape is that of a biconcave disc with a 8.1 micron diameter, 2.3 micron maximum thickness, and a 1-micron thickness in the center. The erythrocyte membrane is composed of an incomplete listing of various components such as simple and complex lipids, proteins with or without enzymatic activity, glycoproteins, metal ions, etc.; its structure is still not fully known (1).

Lipids play an important role in structure and function as far as membrane biochemistry is concerned. Recent estimates of the thickness of the human erythrocyte membrane place it in the range of 150-300 Å (2). Various electron microscopic investigations have suggested that 75 Å is occupied by lipids, perhaps in the form of a semicontinuous leaflet (3). This general picture is supported by data on the quantity and distribution of lipid in the erythrocyte. The lipid in the red blood cell is located in the peripheral region (4).

Under physiological conditions of pH and ionic strength, only anionic groups are detectable on the surface of the erythrocyte by electrophoretic methods (5). It has been demonstrated by different

methods that the negative charge on the red cell surface can be ascribed almost entirely to the carboxylic group of neuraminic acid since neuraminidase-treated red blood cells show a great reduction of electric mobility in an electrical field (6, 7, 8). Also, the colloidal iron staining method shows that positively charged colloidal iron particles do not attach to just specialized areas on the surface of red blood cells, since a layer of the iron particles attach uniformly over the entire surface of the cells (9).

From the fact that red blood cells carry a net negative charge, one would automatically think that red blood cells should repel each other in a physiological suspension. But microscopic investigation shows that one can find rouleaux formation by erythrocytes--aggregation of red cells in the form of a roll of coins--when the blood flow rate is reduced to a low level (10, 11). This aggregation causes the suspension-stability of non-flowing blood to be low. One may argue that surface tension plays an important role when two particles in a suspension come close enough to each other so that the interfaces between each one of them and the fluid will join to one continuous interface and thus will press the particles together.

Some workers observed that high molecular-weight proteins such as fibrinogen, or high molecular weight dextran, strongly pro-

moted rouleaux formation (12). Electron microscopy shows the fibrinogen molecule to be about 500 Å long with a central and two terminal balls, each about 65 Å in diameter. The red cell membrane consists of contiguous craters having diameters in the same range of size (13). It was therefore suggested that the fibrinogen molecule was adsorbed "end on" into the crater-like sites by one of its terminal balls, leaving the other terminal ball projecting outward for subsequent engagement with an adsorbing site on the membrane of a neighboring cell (13).

However, it is known that both red blood cells and proteins carry a net negative charge on their surfaces. Some workers suggested that calcium ions were possibly involved in red blood cell adhesion, serving as bridges between red blood cell and fibrinogen; some workers even suggested calcium ions bridged between two red blood cells (14, 15, 16). To investigate these suggestions, experiments were done to show that the effective binding energy for calcium ions to the carboxylic groups present in the cell peripheral region was very low (17, 18). Other experiments showed that rouleaux formation still took place even in erythrocyte suspensions treated with disodium EDTA, which chelates the free calcium ions in physiological fibrinogen suspensions (19).

One possible mechanism of rouleaux formation is that some as yet unidentified sites on the surface of red blood cells carry positive charges, and these sites attract one end of the fibrinogen molecule by electrostatic force; the other end of the protein molecule is free to be bonded on a neighboring red blood cell.

The purpose of this thesis is to search for these positively charged sites on the surface of red blood cells. Red blood cells obtained by venipuncture are washed with isotonic saline and fixed with 2% acetaldehyde solution. These acetaldehyde-treated red blood cells retain their electrokinetic behavior in the pH range where the untreated erythrocytes are stable (20) and in non-isotonic solutions in which untreated erythrocytes are not stable. The increase in stability of acetaldehyde-treated red cells is thus utilized in the studies of positive sites involving inorganic hydrosols which require non-physiological conditions.

Hydrosols such as silver iodide and ferric oxide prepared in both positively and negatively charged forms are suspended with acetaldehyde-treated erythrocytes. The reasons for this procedure are: (1) Negatively charged colloid particles will adhere to the sites where positive charge exists. (2) Positively charged colloid particles will adhere to all parts on the surface of an erythrocyte which are negatively charged. By means of scanning electron micro-

scopy, one can easily find out the possible location of positive sites on the surface of red blood cells by seeing where the colloidal particles adhere to the red cell surface.

The preference of using scanning electron microscopy to that of transmission electron microscopy is because the scanning electron microscope affords a unique way to look at the external surfaces of red blood cells without any disturbance to the surfaces by the thin sectioning method or replication technique necessary for the transmission electron microscopy (21). Scanning electron microscopy also gives advantages including convenience of sample preparation, large depth of field, an impression of three dimensions, large specimen area, and wide magnification range.

EXPERIMENTAL PROCEDURES

A. Preparation of Monodispersed Silver Iodide

A monodispersed sol may be defined as one in which all particles have exactly the same size and shape. The theory of monodispersed sol production was proposed by La Mer and his co-workers (22, 23). Their theory can be explained as follows: Consider a reaction which continuously generates molecules of a dispersed phase; for example, the proposed mechanism involving the AgI-KI complex:

$$AgI_3^{2-} \rightleftharpoons AgI_2^- + I^-$$

$$AgI_2^- \rightleftharpoons AgI + I^-$$

The continuous dissociation of AgI_3^{2-} to AgI by continuously adding water to the complex system will continuously generate AgI. The concentration of AgI increases steadily, passes the point of saturation A as shown in Figure 1, and reaches a point B at which point the rate of self-nucleation becomes appreciable; as D is approached the rate of nucleation rises very rapidly. However, if nucleation is sudden and occurs in an outburst manner, and the rate of production of new AgI molecules is slow, the region of nucleation (II in Figure 2) is restricted in time and no new nuclei are formed after the initial outburst. The nuclei produced will grow uniformly by a diffusion process (region III in Figure 2) and a sol of monodispersed particles is obtained.

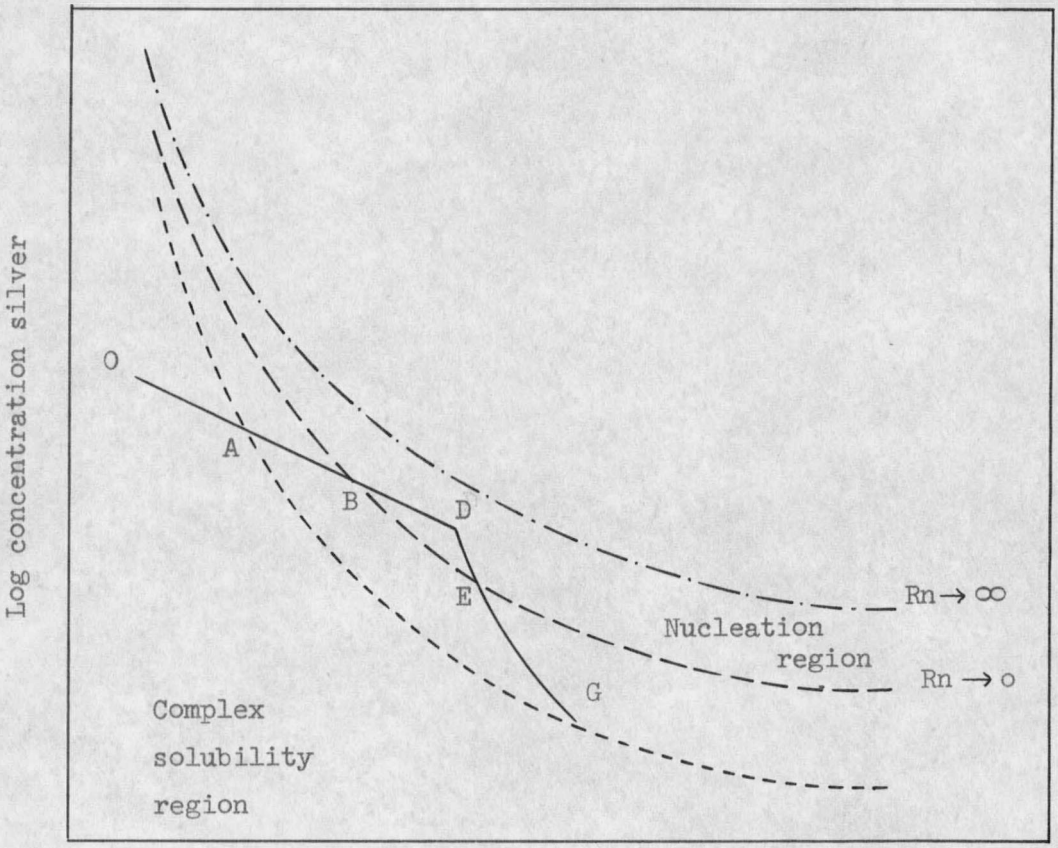

Figure 1. Schematic representation, following La Mer, of conditions for the preparation of monodispersed silver halide sols: .-.-.-. critical limiting supersaturation curve, — — — critical supersaturation curve, ------ solubility curve. Rn = rate of nucleation.