Section III

THE DISCOCYTE - ECHINOCYTE TRANSFORMATION
OF THE HUMAN RED CELL:
DEFORMABILITY CHARACTERISTICS

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Ever since its first description some 80 years ago [11], the shape change of the human red cell from a smooth biconcave disc into a crenated sphere, a phenomenon recently designated as the discocyte-echinocyte transformation [2], has continued to puzzle many hematologists and cell physiologists.

In general, investigators have attempted to gain some understanding about a common mechanism underlying this phenomenon, based on the fact that a multiplicity of agents or conditions are all similarly capable of inducing this somewhat stereotyped response on the part of a red cell [16]. Most popular theories advanced at present to explain the effect of these various agents include a modification in the surface tension of the cell membrane [4], or a shift in its electrostatic equilibrium [18], or even, as proposed more recently, a conformational change in some of its chemical constituents depending either on the ionic environment [8] or on a critical ratio between ATP, Ca++ and Mg++ inside the cell [21].

From a purely mechanical standpoint however it is quite evident that, during the discocyte-echinocyte transformation, a new equilibrium of forces must be achieved at the cell’s surface in order to account for the observed change in geometry. In the last ten years’ literature, few reports only have dealt with the actual documentation of the mechanical behavior of the red cell in relation to this shape change [4, 5, 6, 7, 19].

The present work was intended primarily at evaluating both surface and overall deformability of the human red cell in each of these two

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metastable configurations, making use of a micropipette elastimetry technique originally described by MITCHISON and SWANN [13] and later applied to the red cell by RAND and BURTON [17] and by LACELLE [12]. The objective was first to document whether or not any measurable change in membrane or surface deformability could be detected with this method, and second, to see what would be the effect, if any, of spicule formation on the overall rheological behavior of the cell as it becomes an echinocyte. The last question is of wider hematological interest, considering that several clinical conditions are known to be associated with the presence of spiculated red cells of various types in vivo.

MATERIALS AND METHODS

Figure 1 is a scanning electron micrograph of an echinocyte type-III according to Bessis’ classification [3]. This generally spherical cell with 30 to 40 regularly spaced spicules is the model which will thereafter be referred to as the echinocyte.

This shape configuration can usually be reversed, but this reversibility may be immediate or slow depending on the type of agent used to induce it in the first place. One must therefore distinguish between agents external to the red cell or within the cell itself. Three different external agents were employed in the present experiments to produce echinocytes: 1) alkalinisation of the suspending medium to a pH of 8.5; 2) the effect of 24 hour-incubated plasma, whose echinocytogenic potential is thought to be related to the presence of lysolecithin in sufficient concentrations [9] and, 3) by addition to the suspending medium of minute concentrations of sodium oleate, one of many free fatty acids capable of inducing the shape change. The effect of all three of these agents could be immediately reversed by resuspending the cells in fresh plasma.

The only well known intrinsic action capable of transforming discocytes into echinocytes is that associated with a decrease by 50% or more in the normal ATP content of the red cell [14]. Reversibility in this case can only be achieved by re-incubating the cells with adenine nucleotides for a sufficient period of time, to permit regeneration of the ATP. This type of echinocyte was also obtained by incubating washed red cells in buffered saline for 20 hours at 37°C resulting in a net decrease in their mean ATP concentration of about 80%, as evaluated by the firefly bioluminescence assay [1].

Discocytes were obtained by suspending fresh red cells in a Tris-Sodium Chloride buffer at pH 7.4 to which 1% human serum albumin was added. All cells in this study originated from the same donor and were examined in the fresh state between glass slides and coverslips held one millimeter apart.

Deformability measurements were of three types. Membrane deformability was first evaluated as the pressure « P » in millimeters of water required to induce a standard one-micron penetration of the cells' surface into a 1.5 micron diameter glass pipette (fig. 2). Care was always taken during these measurements that the portion of the cell remaining outside the pipette was not spherical and that it could be aspirated further inside without exhibiting a sudden rise in resistance. A second method for evaluating surface deformability was to measure the distance of penetration « D » in microns of a portion of the cell into the same 1.5 micron pipette, working this time at a fixed pressure differential of 3 mm, of water. Figure 3 finally shows what was called the « Pt » measurement and considered an evaluation of total cell deformability, that is of its actual ability, regardless of specific influences, to traverse a cylindrical channel smaller that its own diameter. In this case, each cell is entirely drawn into a 2.8 to 3.0 micron pipette at a constant rate of pressure application corresponding to 8 mm of water per second.