MEMBRANE FRAGMENTATION AND Ca++-MEMBRANE INTERACTION: POTENTIAL MECHANISMS OF SHAPE CHANGE IN THE SENESCENT RED CELL (*)

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SPECULATION concerning the mechanisms responsible for normal erythrocyte destruction dates back at least to Ponfick's observations [18] of damaged cells. The systematic studies by Rous [22] clearly implied progressive intrinsic alteration of the cell as an important determinant in the normal fate of the erythrocyte and confirmed the earlier suggestion that the spleen was of particular importance in erythrocyte destruction. Currently it is considered that the normal erythrocyte's life span is a function of time dependent changes of the erythrocyte which adversely affect its capacity to survive severe flow requirements of the microcirculation and the phagocytic elements of the reticuloendothelial system.

The mechanical properties of the normal human erythrocyte, determined by the advantageous surface area to volume relationship, fluidity of its contents and the thin viscoelastic membrane capable of isochoric bending, permit it to assume a continuously varying infinite variety of shapes to conform to the requirements of a microcirculation whose capillary channels are less than half the diameter of the cell itself. Figure 1 illustrates graphically the mechanical disadvantage encountered by an erythrocyte which has lost the discocyte configuration. Because the spherical cell (B) is relatively limited in capacity to change its volume confi-

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Configuration (shape) to conform to the aperture as compared to the disco-
cyte (A) and since any attempt to increase the surface area to permit
additional shape change induces tension in the membrane, the spherical
cell behaves as a mechanically rigid body simply because of the minimum
surface area to volume relationship. The membrane possesses viscoelastic
properties [11]; alterations in these physical properties would be expected
to alter the cell's rheologic characteristics. Time related extrinsic factors

which reduce cellular deformability by increasing sphericity and/or modi-
fiying the membrane may be determinants of the cell's life span. These
studies examined the result of in vitro fragmentation of the erythrocyte
membrane and age-dependent alterations of cellular ATP, DPG and Ca++
content, Ca++ permeability and their effects on erythrocyte shape and
deformability to predict whether these factors may contribute to the regu-
lation of the cell's life span.

Rous' experiments [22] led to the conclusion that normal erythrocytes
undergo in vivo loss of membrane by mechanical fragmentation, without
gross loss of hemoglobin, and that such fragmentation contributes to the
cell's ultimate fate. To test this hypothesis, an in vitro fragmentation
technique permitting controlled fragmentation of individual erythrocytes
has been developed [14], as depicted in figure 2. Normal erythrocyte
deformability of the individual cell was first determined as the negative

\[ \text{Fig. 1.} \]

Diagrammatic representation of the cellular deformation required for
erthrocytes to pass through a narrow aperture. A, a normal erythro-
cyte readily deforms to adapt to a 3 \( \mu \) aperture, but spherical cell B, with reduced area/volume relation-
ship cannot deform sufficiently to transit.

\[ \text{Fig. 2.} \]

Technique for controlled in vitro fragmentation of normal human erythrocytes.
Deforming pressure, \( P_t \) (see text for method of measurement), is measured in
the 2.8 \( \mu \) micropipette at the left then, shown on the right, the cell is held while
a portion of membrane is drawn into the 1.2 \( \mu \) micropipette until fragmentation
occurs. After it spontaneously reseals, the \( P_t \) of the same erythrocyte is again
measured to determine the change in deformability caused by the fragmenta-
tion loss of membrane.