# ERYTHROCYTE DEFORMABILITY: CLINICAL ROLE, AND EVALUATION OF DIFFERENT METHODS

THESIS

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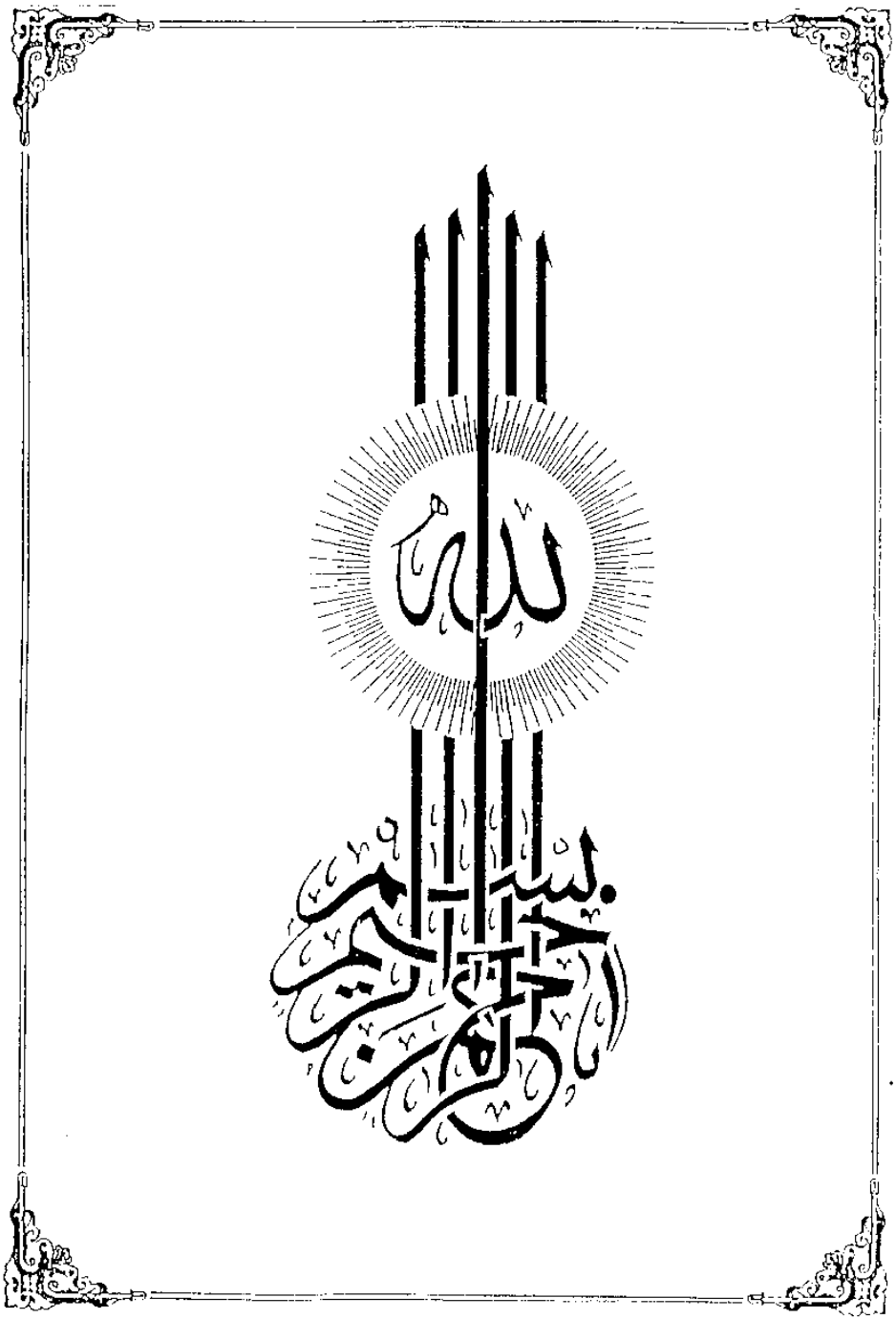
*To my Family*



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# INTRODUCTION AND AIM OF WORK

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Rheology, the science of the deformation and flow of matter, has become of considerable interest to haematologists (Stuart and Kenny, 1980).

All fluids resist, to a greater or lesser extent, attempts to alter their shape, and this resistance to flow is a measure of fluid's viscosity. During flow as layers of fluid move parallel to one another at different rates, a velocity gradient forms between these layers, and this is known as the shear rate, it is measured in reciprocal seconds "$S^{-1}$". The force required to produce this velocity gradient is the shear stress, and is measured in newtons per square meter "$Nm^{-2}$". Viscosity can now be redefined as the ratio of shear stress to shear rate. The unit of viscosity being the pascal second (pas; conversion factor - 1m Pa s = 1 centipose) (Stuart and Kenny, 1980).

Simple fluids, such as plasma and most oils, show a linear relationship between shear stress and shear rate (Newtonian behaviour), so that the viscosity remains conctant.

Whole blood, however, behaves as a "Non-Newtonian fluid" in that viscosity increases exponentially at the low shear rates (below 50 $S^{-1}$) that characterise venous flow. This increase is due to the larger molecular weight plasma proteins (fibrinogen and certain globulins) which overcome the zeta potential between erythrocytes and form rouleaux, these large collular aggregates cause a disproportionate increase in viscosity. At, the high shear rates characterestic of arterial

flow (above 100 $S^{-1}$), rouleaux are dispersed, and individual erythrocytes are deformed into ellipsoids with their long axes aligned in the direction of flow, (Schmid-Schönbein and Wells, 1969; Goldsmith, 1971), thus viscosity is relatively low and virtually constant at high shear rates and is independent of the plasma fibrinogen. In capillaries, the appropriate shear rate range (corresponding to blood flow in) is difficult to determine, since flow may be intermittent and once blood has become stationary, a relatively large force (the yield stress) is required to restart movement. Thus there is uncertainty over the shear rates that characterise capillary blood flow and of the importance of measuring yield stress. For the above reasons, viscosity should always be measured over a wide range of shear rates, but a number of other factors affecting viscosity must also be taken into account whenever viscosity is measured (Stuart and Kenny, 1980).

**Eryhrocyte Deformability (E.D.):**

E.D. or the capacity of the cell to change its shape under applied stress, is important for erythrocyte function, because the small vessels of the microcirculation have a diameter less than that of the resting erythrocyte, cells must deform markedly as they circulate (Mohandas, et al., 1980).

**Importance of E.D.:** It is important for:

- Normal flow of blood (Branemark and Bagge, 1977) particularly at points of capillary bifurcation (Klug et al., 1974).
- Travelling through or passage of RBCs in the smallest capillaries to bring oxygen to the tissues (Groner et al., 1980).

- Impaired E.D. can cause a reduction in cell survival (Weed, 1970; Mohandas et al., 1979).
- E.D. is a physiologically important and characterestic property (Groner et al., 1980).

Such deformation includes their linear alignment as ellipsoids, with rotation of their membranes, during arterial flow (Fischer et al., 1978) and also folding of the cells to produce slipper and other forms in the microcirculation (Gaehtgens et al., 1980).

**Factors that determine ED:**

There are 3 main factors that determine red cell deformability:

1. The viscoelasticity of the cell membrane which in turn is dependent on the molecular structure of the membrane and the metabolic state of the cell.

2. The cell geometry or more specifically the ratio of surface area to cell volume S/V.

3. The internal viscosity of the cell, as determined by the physical state of the haemoglobin (Stuart and Kenny, 1980).

The in vitro measurement of deformability has become a well-accepted means of evaluating red cell viability in vivo (LaCelle, 1969) as well as a useful probe for detecting subtle abnormalities in the structural and functional integrity of the erythrocyte membrane (Evan and LaCelle, 1975).

**Aim of Work:**

A concised review to understand the role of erythrocyte deformability in various clinical disorders, together with evaluation of the different techniques used for its measurement will be done.

# *EVALUATION OF DIFFERENT METHODS FOR MEASURING ERYTHROCYTE DEFORMABILITY*

## EVALUATION OF DIFFERENT METHODS FOR MEASURING ERYTHROCYTE DEFORMABILITY

Blood rheology was mainly concerned with the clinical value of measuring whole blood viscosity (Stuart and Kenny, 1980). Five years later, rheological measurements in clinical studies are more often concerned with the individual components of blood (erythrocytes, leucocytes, platelets and plasma) that collectively influence whole blood viscosity. It is therefore appropriate to review the residual role of measurements of whole blood viscosity while concentrating on new developments in the study of erythrocyte rheology (Stuart, 1985).

### A. MEASUREMENT OF WHOLE BLOOD VISCOSITY

**Whole Blood Viscosity:**

There are many types of viscometers used for measuring viscosity:

- Capillary viscometers (Thurston, 1976).
- Rotational cone-and-plate system (Copley et al., 1975).
- and cylindrical bob-and-cup system (Stuart and Kenny, 1980).

Blood viscosity should be measured at a known and constant shear rate, and the whole of the specimen should be exposed to the same shear. So, capillary viscometers which have a high and inconstant shear rate should not be used (Stuart and Kenny, 1980).

As previously described, whole blood viscosity, must be measured at different shear rates:

**1. At higher shear:**

Most rotational viscometers operate with a low coefficient of variation. The first widely available commercial viscometer of this type was the wells - Brookfield microviscometer Fig. (1). It is simple to operate, but is not sufficiently sensitive to give accurate readings below 20 $S^{-1}$ (Stuart and Cenny, 1980).

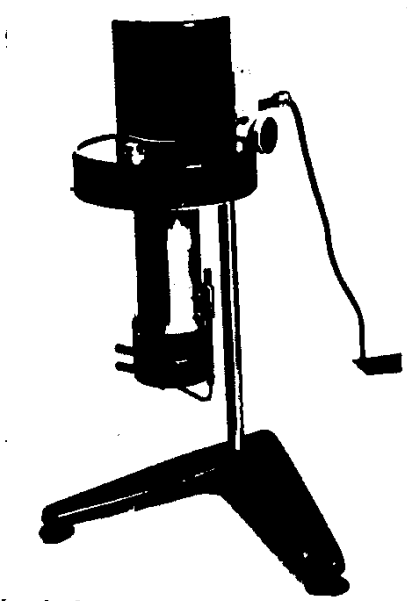

**Fig. (1): Wells-Brookfield whole blood viscometer.** Quoted from (Stuart and Kenny, 1980).

**2. At lower shear:**

The low shear 30 contraves viscometer can be used, Fig. (2) its degree of sensitivity is such that viscosity can be measured at shear rates below 0.1 $S^{-1}$ but it needs:

- antivibration table.

- Protection by wind-shield
- and needs special skill for operating (Stuart and Kenny, 1980).

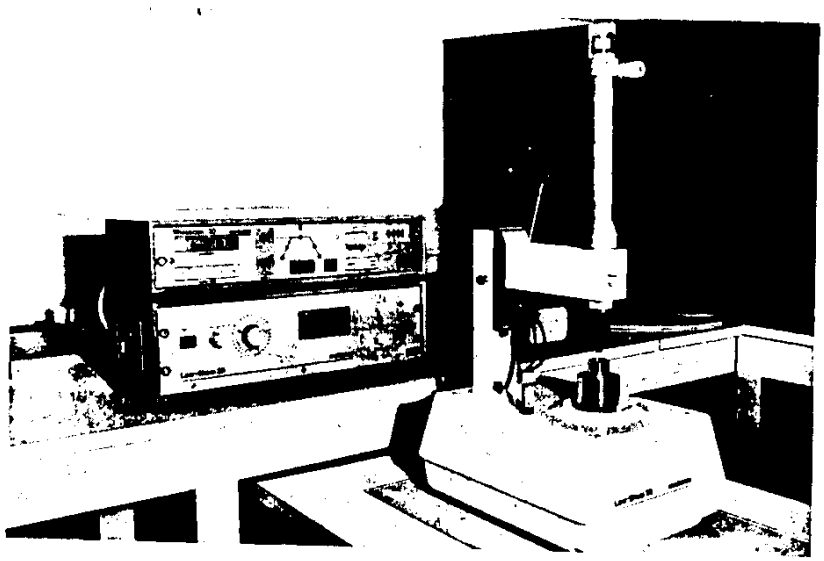

**Fig. (2): Contraves-low shear whole-blood viscometer.** (Quoted from Stuart and Kenny, 1980).

These instruements measure the viscosity by using a known shear rate and the shear stress (force) for the blood sample is recorded then by using the following equation viscosity can be calculated.

Viscosity = shear stress/shear rate. (Stuart and Kenny 1980).

This measurement still has a role as a global test, but its relatively poor sensitivity and specificity, and overdependance on the packed cell volume, have limited its clinical usefulness, and the current

trend is very much towards the rheological study of individual blood components (Stuart, 1985).

**Measurement of Erythrocyte Deformability:**

The principles of deformability and the wide variety of available methods for its measurement have recently been reviewed (Bull, Stuart and Vague, 1984; Stuart, Bull and Vague, 1984). Of these methods, only two have been widely applied clinically:

- Filtration of erythrocytes through pores of 3-5 µm diameter.
- The measurement of erythrocyte elongation using laser diffractometry, (Stuart, 1985).

Less commonly used methods are:

- Aspiration of cells into micropipettes.
- Viscometry of cell suspensions.
- Single Erythrocyte Rigidometer.

and - Cell Transit Time Analyser.

## B. ERYTHROCYTE FILTRATION

**Principle:** Under standard conditions whole blood is passed through a filter with a pore diameter less than that of erythrocyte. The speed of flow is determined largely by the deformability of individual red cells (Reid et al., 1976).

**1. Whole blood filtration:** "Negative pressure system".

Whole blood is forced to pass through a filter by a simple hydrostatic apparatus "Suction mechanism" (Fig. 3), under special conditions,