STUDIES OF THE PHYSICAL PROPERTIES OF THE HUMAN ERYTHROCYTE: MECHANICAL FRAGILITY.

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PART I.

There are few quantitative studies reported in the literature concerning the effects of mechanical trauma on human red blood cells. One of the earliest papers was by Meltzer and Weck (1884). In this they recorded observations made when ox blood was shaken on a bottle shaker for periods of hours and in some cases for days. Their results are extremely difficult to interpret because the suspending medium used was 0.6 per cent. saline, and in addition accurate haemoglobinometry was not possible at that time. Later, in studies pioneering the development of the modern blood bank, Rous and Turner (1916) investigated the effects of shaking samples of stored blood. Their study suffered from the limitation, however, that serial observations of haemolysis with respect to time were not made. Damashek and Miller (1943) in their study of the effects of antibody on the normal erythrocyte used as one of their criteria ability to withstand shaking, while Stats (1943) observed the effects of shaking on the haemolysis of cold agglutinated erythrocytes. An appreciation of the significance of the mechanical fragility test motivated Shen, Castle and Fleming (1944) to reinvestigate the problem and to introduce a mechanical fragility apparatus of somewhat novel design. By their technique accurately reproducible results were possible, and they recorded preliminary observations on the mechanical fragility of erythrocytes in certain haemolytic states. Since this study many papers have been published concerning the application of this particular procedure to the investigation of patients with haemolytic disease.

On examining the technical aspects of the mechanical fragility procedure it is evident that two basic methods of delivering trauma have been utilised. On the one hand, and reaching its highest development in the hands of Damashek and Miller, is the procedure whereby the red cell suspension is vigorously shaken in a test-tube containing glass beads. Here sudden alteration in velocity of both the test-tube and glass beads is the traumatising force. On the other hand, Shen et al. caused the cell suspension to be rotated about the interior of an Erlenmeyer flask containing glass beads, thereby relying on the smooth rolling of the beads over the cells to produce the trauma.

While there are numerous reports of increased red cell fragility in cases of haemolytic anaemia, it has not been possible to draw any close correlation between the degree of fragility and the degree of haemolysis. A critical analysis of this discrepancy reveals that the qualitative aspects of the delivery of trauma may be an important factor. In addition, the quantity of trauma delivered, i.e., the time through which the test is
performed, may be of extreme importance. For example, in the original method of Shen et al., the per cent. haemolysis was read at the end of 80 minutes. It is possible for the degree of lysis to be within normal limits at this time and yet to be very abnormal if the test were continued for a longer period of time. Such in fact has been found in many instances. Thus a careful re-evaluation of the procedure seemed indicated with a view to the development of a technique capable of yielding reproducible results from an analysis of a significant part of the red cell sample both as a tool for the investigation of cases of haemolytic disease and as a test system for the study of the in vitro effects of lytic substances on the physical stability of the erythrocyte.

**Materials and Methods.**

A. All the experiments reported in this paper were performed on normal adult male blood, collected into "Wintrobe" double oxalate solution.

B. The mechanical fragility apparatus used was developed from an old record player with "rim drive". The motor spindle was filed down so that the turntable rotated at about 35 revs. per minute. Twelve clips of the billiard cue type were affixed to the turntable by means of 1½ inch corner irons in the manner depicted in Fig. 1. These clips were large enough to grip the neck of a 50 c.ml. Erlenmyer flask securely. With this arrangement the flasks were fixed about the periphery of the table so that the long axis of each flask was perpendicular to the diameter of the table. The record player was then mounted on the side of a box with the following dimensions 14 inches x 20 inches x 24 inches. A 100 watt light bulb was placed at the bottom of the box and was used to maintain the temperature of the air inside the box at 37° C., as read from a thermometer placed opposite the centre of the turntable. The rotation of the table with the flasks in position resulted in adequate mixing of the air within the box. Before a test was performed care was taken to ensure that the temperature within the box was constant at 37° C.

In practice, it has been found best to control the speed rotation at about 35 revs. per minute. With speeds greater than this, centrifugal force becomes a complication; for under these conditions, the cell suspension is no longer drawn about the side of the flask and no film is formed. In this case the amount of lysis produced falls to zero.