Erythrocyte mechanical fragility test

R. G. COOPER, R. A. KAHN, C. N. CORNELL, and M. E. MUHRER From the Departments of Agricultural Chemistry and Physiology of the University of Missouri, Columbia, Missouri, USA

With the intensive development of cardiovascular prosthetic devices and extracorporeal circulation a need has arisen for a simpler, less time-consuming measure of the mechanical fragility of erythrocytes. The following is a method that will show differences among individuals and in individuals at different times. The test was developed so that a quick but accurate method would be available to measure fragility of red blood cells when studying the effect of mechanical and hydraulic factors on blood.

MATERIALS AND METHODS

A stock supply of Celite 560\(^1\) was prepared by washing the Celite in 5\% hydrochloric acid and then decanting it. Celite, a diatomaceous silica, was obtained from Johns Manville Co., USA.

Received for publication 4 January 1968.

Micro modification of the dilute blood clot lysis time for determining fibrinolytic activity—concluded.

A surface which might lead to artefactual activation of the coagulation and fibrinolytic mechanisms (Niewiarowski and Prou-Wartelle, 1959; Iatridis and Ferguson, 1961) and strict observance of a cold technique prevents the deterioration of plasminogen activator (Fearnley et al., 1957). It can be seen that the difference in dilution of the blood in the two tests (1 in 10 in the standard method and 1 in 11 in the micro modification) does not influence the results significantly. The tests have given similar results in normal human individuals in whom the fibrinolytic system has been activated by exercise. We have also found the micro test to be consistently useful in detecting fibrinolytic activity induced by injecting urokinase or streptokinase into mice and rabbits.

We are grateful to Miss J. Anstead and Miss J. Cowley for technical assistance and to the British Heart Foundation for financial support.

REFERENCES


12 times with distilled water. The washed Celite was then dried at 60°C.

Using a plastic pipette, 1 ml of blood containing citrate anticoagulant (1/10th volume, 3.8%) was placed into each of three 12 × 75 mm polyvinyl chloride tubes containing 60 mg of Celite. An additional aliquot of 1 ml was placed in a tube without Celite for a control. The caps were affixed to the Celite-containing tubes which were then clipped onto the rim of a motor-driven disc of 6 in. diameter (Fig. 1) and rotated at a constant speed of 35 rpm for five minutes.

After rotation the contents of all the tubes were transferred to centrifuge tubes and spun at about 8,000 rcf for about eight minutes. The supernatant plasma was then assayed for haemoglobin by the cyanmethaemoglobin method (Drabkin and Austin, 1935).

This test can be described as 'mechanical' because there is a negligible (less than 0.060 g/100 ml) amount of haemoglobin released from red cells in a sample of blood that is either rotated in a tube without Celite (35 rpm, five minutes), or gently hand rotated in a tube containing Celite (60 mg, five minutes).

### COMMENT

Haemoglobin values were derived by subtracting the

**FIG. 1. Test tube rotator for erythrocyte fragility test.**

*Falcon Plastics, USA, no. 2003.*

*Disc made by Scientific Industries, Inc., USA.*

*Haemoglobin standard and reagent obtained from Ortho-Diagnostics, USA.*

### TABLE I

<table>
<thead>
<tr>
<th>Subject</th>
<th>Tube 1 (g Hb/100 ml)</th>
<th>Tube 2 (g Hb/100 ml)</th>
<th>Tube 3 (g Hb/100 ml)</th>
<th>Mean (g Hb/100 ml)</th>
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<tr>
<td>D.S.</td>
<td>0.466</td>
<td>0.520</td>
<td>0.434</td>
<td>0.473</td>
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<td>D.R.</td>
<td>0.692</td>
<td>0.600</td>
<td>0.640</td>
<td>0.644</td>
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<td>0.412</td>
<td>0.478</td>
<td>0.467</td>
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<tr>
<td>E.H.</td>
<td>0.432</td>
<td>0.426</td>
<td>0.484</td>
<td>0.444</td>
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<td>M.F.</td>
<td>0.710</td>
<td>0.618</td>
<td>0.664</td>
<td>0.664</td>
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<tr>
<td>R.L.</td>
<td>0.432</td>
<td>0.500</td>
<td>0.500</td>
<td>0.477</td>
</tr>
<tr>
<td>B.H.</td>
<td>0.446</td>
<td>0.438</td>
<td>0.544</td>
<td>0.476</td>
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<tr>
<td>G.C.</td>
<td>0.640</td>
<td>0.666</td>
<td>0.600</td>
<td>0.635</td>
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<tr>
<td>R.S.</td>
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<td>0.300</td>
<td>0.322</td>
<td>0.322</td>
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<tr>
<td>S.O.</td>
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<td>0.254</td>
<td>0.286</td>
<td>0.271</td>
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<tr>
<td>E.C.</td>
<td>0.486</td>
<td>0.484</td>
<td>0.506</td>
<td>0.495</td>
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<tr>
<td>G.F.</td>
<td>0.382</td>
<td>0.310</td>
<td>0.304</td>
<td>0.332</td>
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<tr>
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<td>0.564</td>
<td>0.484</td>
<td>0.518</td>
<td>0.522</td>
</tr>
<tr>
<td>L.L.</td>
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<td>0.358</td>
<td>0.378</td>
<td>0.423</td>
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<tr>
<td>J.G.</td>
<td>0.280</td>
<td>0.380</td>
<td>0.306</td>
<td>0.322</td>
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<tr>
<td>J.V.</td>
<td>0.334</td>
<td>0.360</td>
<td>0.346</td>
<td>0.347</td>
</tr>
<tr>
<td>R.K.</td>
<td>0.542</td>
<td>0.620</td>
<td>0.614</td>
<td>0.592</td>
</tr>
<tr>
<td>L.K.</td>
<td>0.638</td>
<td>0.618</td>
<td>0.652</td>
<td>0.636</td>
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<tr>
<td>P.S.</td>
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<td>0.416</td>
<td>0.438</td>
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<td>T.T.</td>
<td>0.540</td>
<td>0.568</td>
<td>0.560</td>
<td>0.556</td>
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</table>

Mean for all samples = 0.476.

SD = 0.118.

### TABLE II

<table>
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<th></th>
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<td>Tube 1</td>
<td>0.542</td>
<td>0.592</td>
<td>0.572</td>
<td>0.534</td>
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<tr>
<td>Tube 2</td>
<td>0.620</td>
<td>0.592</td>
<td>0.532</td>
<td>0.586</td>
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<tr>
<td>Tube 3</td>
<td>0.614</td>
<td>0.630</td>
<td>0.542</td>
<td>0.548</td>
</tr>
<tr>
<td>Mean</td>
<td>0.592</td>
<td>0.605</td>
<td>0.549</td>
<td>0.556</td>
</tr>
</tbody>
</table>
Differentiation of foetal and maternal erythrocytes in formol-fixed tissues

C. G. PAINE From the Department of Pathology, Jessop Hospital for Women, Sheffield

The elution techniques of Kleihauer, Hildegard, and Betke (1957) and of Singer, Chernoff, and Singer (1951), as applied to peripheral maternal blood, have provided valuable methods for determining the severity of trans-placental bleeding. Their application to formol-fixed tissues has not proved successful in demonstrating the sites of admixture of the foetal and maternal circulations. A differential destruction technique applicable to formol-fixed placental tissue is therefore described.

MATERIALS AND METHOD

Full-term placentae were fixed whole in 10% formol saline for four days before representative blocks were cut for section. Tissues were processed in the normal way for paraffin sections and cut at 5μ. Sections were dewaxed and taken through to water. They were then exposed at 37°C to peptic digestion in an acid buffer solution. After washing in water for 10 minutes, they were then stained by Erhlich’s haematoxylin and alcoholic eosin.

SOLUTIONS

1 Pepsin (BPC) 25 units per ml in distilled water
2 Glycine buffer
   A Glycine .................................. 7·505 g
   NaCl .................................. 5·85 g
   Distilled water to 1 litre
   B 0·1 N HCl

Thirty-eight ml Solution A and 62 ml Solution B provided a buffer solution at pH 1·8.

Sections were incubated at 37°C for varying periods in petri dishes containing 1·25 ml pepsin solution and 5·0 ml buffer solution, the final concentration of pepsin being 5 units/ml.

RESULTS

Incubation for periods in excess of 10 minutes resulted in complete destruction of both foetal and maternal erythrocytes. Destruction of maternal red cells was seen to start after four minutes' incubation and was complete after eight minutes. Destruction of foetal red cells was not appreciable until after eight minutes' incubation. At eight minutes a clear distinction between the two types of cell was demonstrable (Figs. 1 and 2). Little change was seen in the histological detail of the remaining tissue even after 40 minutes' incubation.

Received for publication 7 February 1968.

FIG. 2. Plasma haemoglobin in samples of normal human blood.

haemoglobin (g/100 ml) of the control tube from the haemoglobin (g/100 ml) of the rotated tube.

Figures 2 and 3 show the effect of varying the time of rotation or concentration of Celite on the haemoglobin released from samples of normal human blood. In Table I the erythrocyte fragility of samples of normal human blood is recorded. Human subject R.K. (Table II), studied on four different occasions, revealed small variations in erythrocyte fragility.

The amount of blood or Celite and the time of rotation can be varied to improve the degree of precision and to suit the convenience of the laboratory.

This work was supported by USPHS grant no. HE-07181. We are indebted to the Pathology Department of the University of Missouri for technical assistance.

REFERENCE

Erythrocyte mechanical fragility test.

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doi: 10.1136/jcp.21.6.781

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