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

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Methods in general use for measuring circulating fibrinolytic activity include the dilute blood clot lysis time (Fearnley, Balmforth, and Fearnley, 1957) and the euglobulin lysis time (von Kaulla, 1963). This paper gives details of a micro modification of the first of these which can be carried out on capillary blood and has been found useful in demonstrating increased fibrinolytic activity in children, small animals, and in surveys where serial determinations are required.

APPARATUS AND REAGENTS

WHITE CELL DILUTING PIPETTES Standard white cell pipettes are treated with water-soluble silicone solution (Siliclad, Clay Adams Inc. NY). These pipettes are chilled by placing them in a 6 × 1 ½ in. boiling tube immersed in melting ice.

PHOSPHATE BUFFER, pH 7.4 \( \text{KH}_2\text{PO}_4 \), 3-02 g, dissolved in 250 ml distilled water, is added to 9-47 g \( \text{Na}_2\text{HPO}_4 \) dissolved in 1 litre of distilled water. This buffer is stored at 4°C.

THROMBIN-BUFFER REAGENT Bovine thrombin (Leo Pharmaceutical Co.) is added to phosphate buffer to give a final concentration of 5 NIH units of thrombin per millilitre of buffer. This reagent is made up freshly on the day of use and stored at 4°C.

METHOD

A free flow of capillary blood is obtained by pricking a finger tip or ear lobe or heel. Blood is drawn up to the ‘1’ mark of a chilled white cell diluting pipette and diluted with cold thrombin-buffer reagent to give a final dilution of 1 in 11. After rapid mixing the contents of the pipette are blown out into a 3 × ½ in. glass test tube. This tube is placed in melting ice for 20 minutes to allow a clot to form. It is then transferred to a waterbath at 37°C and examined at intervals for lysis. The length of time between placing the clot at 37°C and complete disappearance of the clot is the lysis time.

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Received for publication 19 February 1968.
Erythrocyte mechanical fragility test

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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 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; Iatriidis 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.

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Micro modification of the dilute blood clot lysis time for determining fibrinolytic activity.
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doi: 10.1136/jcp.21.6.780