Measurement of spontaneous fibrinolytic activity

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The spontaneous fibrinolytic activity of blood is due to a labile activator which appears to be stabilized by adsorption to fibrin (Fearnley and Tweed, 1953; Fearnley, 1953; Flute, 1960). At present activator activity can be measured only indirectly through its conversion of plasminogen to plasmin which in turn causes fibrinolysis. Whatever method is used it is essential to cool the blood sample to 0°C. immediately it is obtained, and to carry out any subsequent manipulations at this temperature in order to preserve the activator which disappears rather rapidly from fluid blood kept at room temperature.

Two factors inhibit spontaneous fibrinolysis, the natural inhibitory capacity of the blood, which is probably complex, and the presence of calcium (Fearnley and Tweed, 1953; Fearnley and Lackner, 1955). The first can be diminished by dilution as shown by MacFarlane (1937) or by separation of the euglobulin fraction, the inhibitors remaining in the supernatant. Calcium can be removed by precipitation. It is easier to measure fibrinolytic activity in blood than in plasma, and we have developed two simple methods for this purpose, one of which is really a modification of the other.

METHOD 1: DILUTE BLOOD CLOT LYSIS TIME

This has been described by Fearnley, Balmforth, and Fearnley (1957).

REAGENTS These are as follows:—
Phosphate buffer (p*H* 7·4) Na_2HPO_4, 9·47 g., is dissolved in 1 litre distilled water, and to this is added 3·02 g. KH_2PO_4 dissolved in 250 ml. of distilled water. The buffer is sterilized by heat before use.

Thrombin solution Parke Davis topical thrombin, 5,000 u., is dissolved in 100 ml. N saline. The thrombin solution is conveniently stored in lots of 3·0 ml. in bijou bottles at −15°C. Satisfactory activity is maintained at this temperature for a month.

GLASSWARE Pipettes and test tubes are plugged with cotton wool and all glassware is sterilized by heat.

PROCEDURE Blood (1·0 ml.) is obtained by venepuncture;

0·2 ml. of this is added by pipette to one or more plain glass tubes (10 cm. × 1.5 cm.) surrounded by ice and containing 1·70 ml. phosphate buffer and 0·1 ml. thrombin solution, the latter being added just before the test. Clotting occurs within about a minute. The tubes are placed in a refrigerator for 30 minutes, and then in a waterbath at 37°C. Retraction of the clots begins within a few minutes and at five to 10 minutes the tubes are briskly rotated between the palms of the hands to ensure that the partially retracted clots are freed from the walls of the tubes. Retraction proceeds uniformly, showing no variation with different subjects or with different degrees of fibrinolytic activity, until the clots have retracted to about one tenth of their original size and are floating in the buffer. Lysis is first noticed as a shagginess followed by breaking up of the clot. The end-point is now taken as the breaking up of the clot, rather than its total disappearance, because a small quantity of fibrin and red cells may remain floating on the surface of the fluid for several hours after lysis is virtually complete. Lysis time and fibrinolytic activity are inversely related, so that a long lysis time indicates low activity and a short lysis time high activity.

Figure 1 shows paired clots from six people at various stages of lysis.

The method is surprisingly accurate, the error between duplicates being not more than 10%. One disadvantage is the difficulty of obtaining the
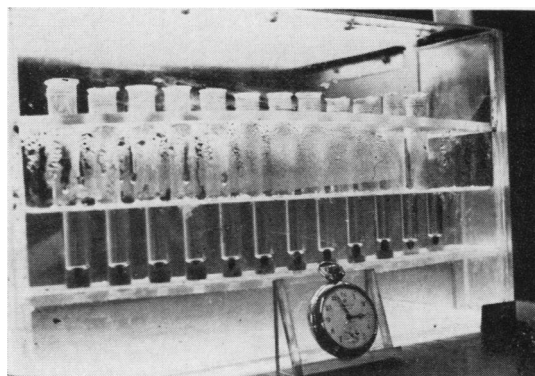

FIG. 1. *The dilute blood clot lysis time. Paired clots from six people at various stages of lysis.*
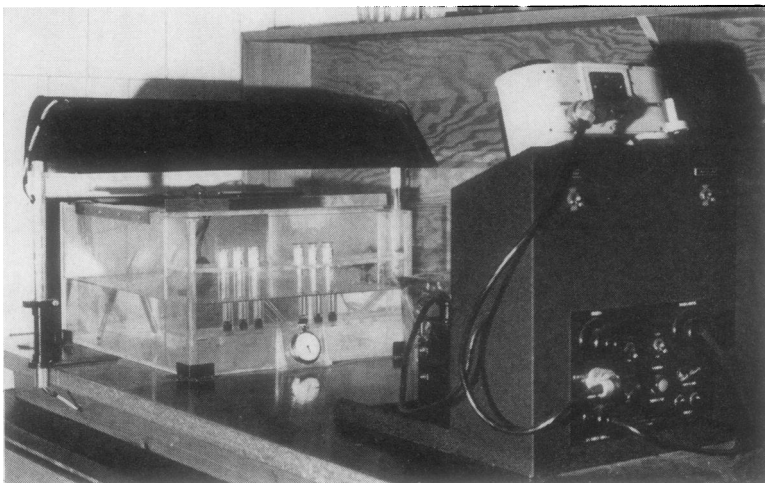
FIG. 2. *The photographic apparatus for recording the end-point of the dilute blood clot lysis time.*

end-point when the lysis time is long, *i.e.*, between 12 and 24 hours. As shown by Lackner and Goosen (1959) this can be overcome by photography. Figure 2 illustrates the photographic apparatus we use.[1]

Billimoria, Drysdale, James, and Maclagan (1959) have designed a modification of the dilute blood-clot lysis time test in which a number of tubes containing clots made from the same dilution of blood are incubated, and the haemoglobin loss is calculated at intervals of incubation. From this a curve is constructed and the 'half' lysis time calculated.

METHOD 2: THE FIBRINOLYTIC POTENTIAL

The method has already been described in this Journal by Chakrabarti and Fearnley (1962).

Blood obtained by venepuncture is immediately cooled in melting ice. Blood, 5 ml., is diluted with an equal volume of ice-cold phosphate buffer, pH 7·4, M 0·12. (for preparation of buffer, see Fearnley *et al.*, 1957). Ten 1·90 ml. volume dilutions of blood in phosphate buffer are made in test-tubes standing in melting ice, the dilutions being such that the final concentration of blood in the test (volume 2·0 ml.) ranges from 5 to 27·5%, the blood concentration interval between the tubes being 2·5%. To each tube is added 0·1 ml. physiological saline containing 5 units of Parke Davis topical thrombin, each tube being shaken after thrombin has been added. After 15 minutes the tubes are placed in a waterbath at 37°C. Five to 10 minutes later each tube is briskly rotated between the palms of the hands to ensure freedom of retraction. The test is read after 12 hours' incubation, the highest concentration of blood to show complete lysis being the end-point.

[1]Made specially by Messrs. Shackman of Chesham, Bucks.

This method depends on the fact that because of inhibition the more concentrated the suspension of blood the longer does lysis take. Consequently the greater the number of tubes showing lysis after 12 hours' incubation, the greater is the fibrinolytic activity of the blood sample.

COMMENT

It will be evident that both methods yield a crude overall picture of blood fibrinolytic activity. Each will be affected by activator level on the one hand and by inhibitor level on the other. The two methods correlate well, but the dilute blood clot lysis time test is the more sensitive.

We routinely use the dilute blood clot lysis time, and have found it to be a useful exploratory tool. It can be used to compare the blood fibrinolytic activity of individuals and to make daily determinations on patients. It reveals diurnal variability and the effects of stress and physical exercise. It is sensitive to the effects of drugs given to increase or diminish the spontaneous fibrinolytic activity of blood.

Nevertheless, in order to amplify information already obtained, exploration in depth seems desirable. The test does not reveal how far differences in fibrinolytic activity are related to activator or inhibitor levels, or to what extent the effects of testosterone, phenformin, and corticosteroids on blood fibrinolytic activity are mediated by changes in these parameters. By combining this test with the euglobulin lysis time on the one hand and with the plasmin inhibition test on the other it may prove possible to obtain such information.

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DR. JAMES, referring to Dr. Billimoria's method quoted by Dr. Fearnley, stated that, while it was true the number of patients they could study was limited to three or four a day, in his experience the method was accurate and reproducible.