The relationship of the dilute whole blood lysis time to the fibrinolytic activity of blood: effect of change in plasma fibrinogen

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SYNOPSIS The rate of digestion of fibrin was measured *in vitro* by an isotopic technique in 140 blood samples of differing fibrinogen concentration; the assessment of fibrinolytic activity thus obtained was compared with a standard method of measurement, the dilute whole blood lysis time. The lysis time was related exponentially to the fibrinolytic activity as measured by the isotopic technique, and further was influenced markedly by alteration in the plasma fibrinogen concentration. The relevance of these observations to the use of lysis time methods for the measurement of fibrinolytic activity is discussed.

Two standard methods of measuring the fibrinolytic activity of blood involve setting up clotted samples of either diluted whole blood (MacFarlane, 1937; Fearnley, Balmforth, and Fearnley, 1957) or the diluted euglobulin fraction of plasma (MacFarlane and Pilling, 1946; Von Kaulla and Schultz, 1958) with subsequent incubation at 37°C and measurement of the time at which the clot disintegrates macroscopically. Difficulties inherent in both techniques include the lack of a precise endpoint and the presence of varying amounts of substrate, ie, fibrin, in the test system. Thus the interpretation of results when the fibrinogen concentration is abnormal or is changing from day to day is complicated by the difficulty of differentiating the effect of change in substrate concentration from change in actual fibrinolytic activity. The introduction of a simple isotopic method for measuring the rate of fibrin digestion *in vitro* (Hickman, 1971) has facilitated further scrutiny of the lysis time methods, particularly with respect to the influence of the plasma fibrinogen concentration and to the relationship of lysis time measurements to the fibrinolytic activity of the blood.

Materials and Methods

GENERAL

One hundred and forty blood samples were obtained

from patients over the age of 50 years in surgical wards: all these patients were receiving ¹²⁵I-labelled fibrinogen for the detection of leg vein thrombosis. The fibrinogen preparation was processed at the Lister Institute from a pool of not more than five donors accredited by the Regional Transfusion Centres as free from the hepatitis carrier state. Iodination was carried out at the Radiochemical Centre, Amersham, Bucks, and the material was presented in freeze-dried form in ampoules containing 0.7 mg fibrinogen and 100 μCi of ¹²⁵Iodine. When this preparation was tested *in vivo* the amount of plasma radioactivity clottable by thrombin was usually between 80 and 93% of the total plasma radioactivity. In the unusual circumstance of a fibrinogen preparation having a 'clottability' *in vivo* of less than 80% it was discarded for use in the fibrinolytic assay since it was found that such preparations introduced inaccuracies into the isotopic measurements. Blood samples were taken with minimal haemostasis and glassware was washed both in acid and detergent.

PREPARATION OF BLOOD DILUENT MIXTURE

Both the isotopic studies and the dilute whole blood lysis times (BLT) employed aliquots of the same mixture of blood and diluent. The mixture was prepared by adding, immediately after withdrawal, 10 ml of whole blood to 85 ml ice-cold acetate citrate diluent, pH 7.4, contained in a conical flask. The acetate citrate diluent was used, both because it increases the rate of fibrin digestion *in vitro*, the citrate ions inhibiting probably factor XIII, and the

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acetate ions inhibiting antiplasmin activity (Gallimore and Shaw, 1969), and because it has been claimed to allow the endpoint to be determined with greater accuracy (Gallimore, 1967). Aliquots of the blood diluent mixture were used for both the BLT and the isotopic measurements.

WHOLE BLOOD AND PLASMA FIBRINOGEN CONCENTRATION: DILUTE WHOLE BLOOD LYSIS TIME AND ISOTOPIC MEASUREMENTS OF FIBRINOLYTIC ACTIVITY

The techniques used were as described previously (Hickman, 1971). The principle of the isotopic method was to measure the rate of fibrin digestion *in vitro*. It was considered that this provided a more direct measurement of fibrinolytic activity than the lysis time methods, and that within normal ranges of activity it was largely independent of the fibrinogen concentration. This method has since been simplified (Hickman and Gordon-Smith, 1972).

FIBRINOGEN ENRICHMENT STUDIES

Studies involving the addition of fibrinogen *in vitro* were performed in order to determine whether the isotopic method of measuring fibrinolytic activity was truly independent of the fibrinogen concentration.

The fibrinogen solution was prepared by dissolving 1g human lyophilized fibrinogen (Kabi Pharmaceuticals Ltd, London, W5) in 100 ml sterile water. Aliquots of this solution were stored at -20°C before use. Duplicate 15 ml blood samples were taken into syringes containing either 2 ml fibrinogen solution or 2 ml 0.9% saline. The fibrinogen concentration, specific activity, and fibrinolytic activity for each sample were then measured using the techniques described above.

Results

Figure 1 shows fibrinolytic activity, as measured by the isotopic technique, plotted against the dilute whole blood lysis time. The results are shown in three groups according to the whole blood fibrinogen concentration. The low range corresponds to a whole blood fibrinogen concentration ranging between 0.81 mg and 1.40 mg per 0.5 ml blood, corresponding to a plasma fibrinogen concentration of 290 mg% to 509 mg% (assuming a PCV of 45%). The intermediate range corresponds to a whole blood fibrinogen concentration between 1.41 mg and 2.00 mg per 0.5 ml blood (equivalent to a plasma fibrinogen concentration between 510 mg% and 727 mg%) and the high range corresponds to ranges of 2.01 mg to 2.60 mg per 0.5 ml blood (plasma fibrinogen 728 mg% to 924 mg%). Such a range of

plasma fibrinogen concentrations is by no means unusual when studies include samples from subjects whose acute phase protein synthesis has been stimulated by factors such as surgical operation or infection. In postoperative subjects the whole blood, as against plasma, fibrinogen concentration is further increased by the tendency for the PCV to fall after operation. Thus the range of fibrinogen concentration will be greater when samples of whole blood are used as in the BLT than when samples of plasma are used as in the euglobulin lysis time.

The relationship between lysis time and fibrinolytic activity is clearly not linear (fig 1). In fact when attempts were made to obtain the highest correlation using different mathematical plots, the logarithm of the lysis time plotted against the logarithm of fibrinolytic activity produced a higher coefficient of correlation ($r = 0.7909$) than either a reciprocal plot ($r = 0.6586$ or a plot of the reciprocal of the lysis time against the logarithm of fibrinolytic activity ($r = 0.6834$). Accordingly, regression lines obtained using the double logarithmic plot have been used to demonstrate the effect of fibrinogen concentration upon the relationship of lysis time to fibrinolytic activity (fig 2). In fig 2 individual regression lines obtained using a double logarithmic plot have been superimposed on the linear plot of the samples grouped according to their fibrinogen concentration as described previously. Each individual correlation coefficient shows a significant improvement (applying Fisher's 'z' transformation) on the overall correlation coefficient, and it will be seen that change in the fibrinogen concentration exerts a marked effect upon the relationship between slope and lysis time. The tendency of the regression lines to merge at the higher range of activity suggests that some degree of substrate enhancement occurs at this range of activity. The fact that, when plotted in this way, the results are grouped according to fibrinogen concentration is suggestive that the isotopic method of measuring fibrinolytic activity, at least within the greater part of the physiological range of activity, is largely independent of the substrate concentration.

Further evidence that the isotopic method is independent of the fibrinogen concentration is obtained from the duplicate isotopic studies in which one series has been enriched by the addition of fibrinogen to the blood diluent mixture. In the 10 studies performed the lysis time was significantly prolonged in each of the fibrinogen-enriched series but with only minor alteration in the slope (see table). An individual study is shown in figure 3.

Discussion

Blix (1961) and Gallimore and Shaw (1969) both

demonstrated that the addition of fibrinogen to blood samples prolonged the dilute whole blood lysis time. Blix further showed that the amount of fibrinogen in the euglobulin precipitate of plasma was determined by the plasma fibrinogen concentration, indicating that the euglobulin lysis time would be affected similarly by change in plasma fibrinogen. It was not possible, however, to quantitate this effect using their *in vitro* methods, and scepticism has been expressed on the validity of the findings based as they were on manipulation *in vitro* of the fibrinogen concentration. The present study in fact confirms

their finding and provides some guidance on the magnitude of the effect exerted by change in plasma fibrinogen. It becomes evident from a study of fig 2 that in situations where the fibrinogen concentration has altered significantly an assessment of fibrinolytic activity by lysis time methods will be difficult to interpret. For example, one can question to what extent the so-called fibrinolytic 'shutdown' occurring after injury (Innes and Sevitt, 1964; Chakrabarti, Hocking, and Fearnley, 1970) is a reflection of the increased fibrinogen concentration rather than an actual reduction of net enzyme

Study	Fibrinogen Concentration (mg/100 ml plasma)		Slope		Whole Blood Lysis Time (hr)	
	Control	Enriched	Control	Enriched	Control	Enriched
1	204	493	0.78	0.76	2	3.5
2	234	465	0.22	0.28	4	6
3	239	442	0.49	0.44	3	4
4	498	748	0.07	0.18	> 24	24
5	475	734	0.75	0.81	3.5	4.5
6	494	689	0.94	0.94	3	4.5
7	200	489	0.82	0.73	2.5	3
8	368	548	0.51	0.52	3.5	4.5
9	553	778	1.64	1.34	1.5	2
10	670	754	0.72	0.55	3.5	6

Table The results in 10 studies of the effect of the addition of fibrinogen in vitro to one of a pair of duplicate samples

Using Student's *t* test there is no significant difference between the slopes (*P* 0.3) but there is a significant difference between the whole blood lysis time groups (*P* 0.001).

Fig. 3 Fibrinogen enrichment study no. 2 is illustrated. The fibrinogen concentration has been increased from 234 mg% in the first sample (●) to 465 mg% in the second sample (△). This has prolonged the whole blood lysis time from four to six hours with only minor alterations in the rate of the reaction.

activity. Certainly the studies of this phenomenon have relied heavily on lysis time methods and it is noteworthy that the 'shutdown' occurs during a period when not only is the plasma fibrinogen concentration raised, but the presence of fibrin degradation products in the serum suggests that the proteolysis of fibrin is actually increased.

However, quite apart from the technical difficulties described above, there are more basic problems involved in assessing the significance of measurements of the fibrinolytic activity of circulating blood. For example, individual measurements of activity obtained by any method can be of only limited significance in view of the wide fluctuation of activity that is present in most individuals. Furthermore, activity in a sample of peripheral venous blood may bear little relationship to activity in other blood vessels or to activity at the vessel wall, and in isolation gives little information on the balance between fibrinolytic enzymes and their inhibitors. Again, that fibrin degradation products appear in the serum at a time when the fibrinolytic activity of blood cannot be shown to be increased and may actually appear reduced is a further interesting anomaly casting real doubt on the validity of this approach to the measurement of fibrinolysis.

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