

Methodology related to the assessment of fibrinolytic parameters

Abstracts

Fibrin Lysis by Thrombin Elevating Levels of Fibrinogen and Fibrin-Related Antigen (FR-Antigen) in Human Serum as Shown by AutoAnalyzer Assay¹

CLARENCE MERSKEY, ALAN J. JOHNSON, AND PARVIZ LALEZARI (Department of Medicine and Unit for Research in Aging, Albert Einstein College of Medicine of Yeshiva University, New York, the Department of Medicine, New York Medical Center, and the Montefiore Hospital and Medical Center, New York)

Antigens reacting to antifibrin antibody have been identified in human serum by a number of immunological methods including electrophoresis, diffusion, and the tanned red-cell haemagglutination inhibition immunoassay.

These antigens are commonly designated as fibrinolytic digestion products (FDP), but since the immunologically reacting material may consist of a mixture of soluble fibrin monomer, fibrinogen, and/or FDP and complexes of these, the term fibrinogen-fibrin-related antigen (FR-antigen) seems preferable. A highly sensitive and reproducible haemagglutination inhibition immunoassay for FR-antigen has now been automated with the use of the AutoAnalyzer.

It has generally been assumed that the addition of fibrinolytic inhibitors, before or during clotting, prevents further *in vitro* lysis so that concentration of these antigens in serum does not exceed that which might have occurred *in vivo*. However, these studies demonstrate a time- and temperature-dependent increase in FR-antigen *in vitro*, despite the addition of fibrinolytic and cathepsin inhibitors, after normal or abnormal blood or plasma has clotted; serum from whole blood contains more than serum from plasma. Highly purified fibrinogen, prepared by a number of different procedures, behaves similarly. Fibrin, rather than fibrinogen, is essential for *in vitro* lysis since in most cases unclotted plasma appears to be unaffected by incubation at 37°C.

The increase in antigen is probably caused by thrombin evolved during clotting and/or added

in vitro. Arvin and Reptilase have an effect similar to thrombin suggesting that splitting of one or more arginine or lysine bonds in fibrin may be, in part at least, responsible for this phenomenon.

To obtain the minimal level of FR-antigen (<0.5 µg/ml), normal plasma is clotted for four hours at 0°C with 5.0 U thrombin/ml, CaCl₂ (0.0125 M), and EACA (0.05M). Slightly higher levels of antigen, probably adequate for clinical diagnosis, are obtained with a 30-minute clotting time of citrate plasma (not blood) at room temperature (not 37°C) when the thrombin concentration is increased to 20 U/ml.

Evaluation of the Original Fibrin Plate Method for Estimating Plasminogen Activators

N. A. MARSH AND C. L. AROCHA-PINANGO (Queen Elizabeth College, London, and St Thomas's Hospital, London)

The fibrin plate has been described as semi-quantitative, complicated, unreliable, and difficult to use, and since its introduction in 1952 by Astrup and Müllertz many modifications have been proposed in an attempt to improve the method. In view of these criticisms, a study was carried out in order to evaluate the original method and some of the subsequent modifications.

SUBSTRATE STANDARDIZATION

Six fibrinogen preparations (three human, three bovine) were analysed for clottable protein, plasminogen, and citrate contents. All three parameters varied widely and polyacrylamide gel electrophoresis showed a considerable degree of heterogeneity in the preparations. These variations demonstrated the need for careful substrate standardization so that fibrin plates could be made with known clottable protein and plasminogen contents.

ADDITIONS TO THE FIBRIN PLATE

Calcium chloride was found to stabilize plates so that they could be stored for at least seven days. Sensitivity was apparently unaffected. Dextran was

¹Supported by USPHS grants nos. HE 06191, HE 10036, HE 05003, and HD 00674.

also found to be a stabilizing agent, but its use made plate preparation unnecessarily complicated.

PREPARATION OF PLATES FOR ASSAY OF PROTEOLYSIS

Heated plates are used frequently to assay proteolysis, but their sensitivity is greatly reduced. As an alternative, the addition of AMCHA to the plate was studied. AMCHA plates were also found to have a reduced sensitivity to proteolysis but were marginally easier to prepare.

ACCURACY OF THE METHOD

A number of urokinase solutions of known strength were assayed on the fibrin plate and by a standard tube clot lysis method. The methods were found to be comparable in accuracy although at low activator concentrations the plate became less reproducible.

The original fibrin plate was found to be a convenient and reliable method for assaying plasminogen activators. The only useful modification was the addition of calcium chloride for fibrin stabilization. For immediate use, the unmodified plate was quite acceptable.

Comparison between a Direct and an Indirect Method for Determining Plasminogen

INGA MARIE NILSSON AND ULLA HEDNER (*Coagulation Laboratory, Allmänna Sjukhuset, Malmö, Sweden*)

An immunochemical method for the determination of plasminogen has been available since 1968 (Ganrot and Niléhn, 1968). As this assay is quite simple and rapid and thus would be of advantage in laboratory work, we considered it of interest to compare it with the caseinolytic method for determining plasminogen recommended by Johnson, Kline, and Alkjaersig (1969). The immunochemical method we used was a slight modification of the method of Ganrot and Niléhn (1968) by Ekelund, Hedner, and Nilsson (1970). To avoid activation of plasminogen *in vitro* the blood was collected in tubes containing EACA. The determinations were made according to Laurell (1966) with electrophoresis in agarose gel containing a specific rabbit antiserum to human plasminogen. The serum samples diluted 1:5 migrates into the gel and precipitation peaks are formed. The height of these peaks obtained is a measure of the amount of antigen in the samples. Mixed serum from 20 apparently healthy volunteers was used as standard. The normal range was found to be 75-137% (44 normal persons examined).

In the caseinolytic method for determining plas-

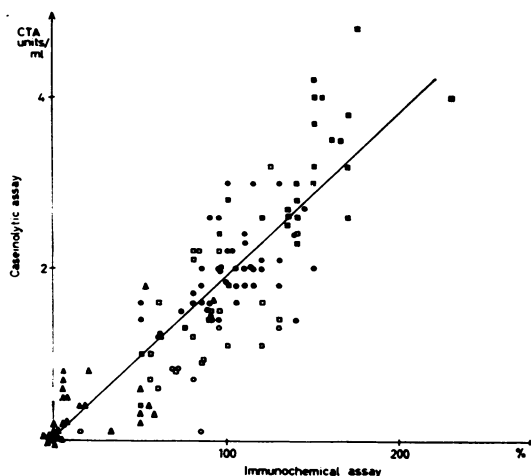


Fig. Comparison of plasma plasminogen levels assayed by caseinolytic and immunochemical methods.

minogen (Johnson *et al.* 1969) the plasminogen in plasma was precipitated by 30% ammonium sulphate. After removal of the supernatant containing the plasmin inhibitors the precipitate was resuspended and its plasminogen content was determined with a caseinolytic method. Removal of the plasmin inhibitors in this way is claimed to be more effective than by acidification. The results were expressed in CTA units. Among 44 normal individuals the mean value was found to be 1.9 CTA units/ml (SD \pm 0.46) and the normal range 1.0-2.8 CTA units/ml.

Plasminogen determinations were performed with both methods in 44 normal subjects (aged 18-45 years), 25 pregnant women in the third trimester, seven patients with septicaemia, nine foetuses obtained by legal induced abortion, 24 patients with liver diseases, and 20 patients during thrombolytic therapy.

In all 129 subjects the plasminogen values varied as measured with the caseinolytic method between 0 and 4.8 CTA units/ml and as measured with the immunochemical method between 0 and 230%. From the figure it is apparent that there was a close correlation ($r = 0.9$; $P < 0.001$) between the plasminogen values determined immunochemically and as caseinolytic activity both for high and low plasminogen levels.

The immunochemical plasminogen assay has proved reproducible and as sensitive as the caseinolytic method. Inhibitors of plasminogen activation do not influence the results.

¹This investigation was supported by grants from the Swedish Medical Research Council (B71-19X-87-07A)