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 Assay[1]

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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, $CaCl_2$ (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.

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Evaluation of the Original Fibrin Plate Method for Estimating Plasminogen Activators

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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 heterogenisity 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