J. clin. Path. (1964), **17**, 313

A method for detecting and estimating plasminogen in cerebrospinal fluid

R. L. NEWMAN

From Queen Mary's Hospital for Children, Carshalton, Surrey

Fibrin is still deposited in the ventricular system as a complication of meningitis even when the infecting organism is eliminated by adequate chemotherapy, and its presence may also raise problems in neurosurgery. Intravascular thrombi can now be lysed by activation of the plasma fibrinolytic system and we therefore thought it worthwhile to see if a similar system existed in the cerebrospinal fluid and, if so, whether it could be activated in a similar way. In this event, the deposition of fibrin might be preventable or fibrin deposits lysed.

The essential points of enquiry are the existence of plasminogen in the cerebrospinal fluid and the presence of inhibitors of anti-plasmin type, and we decided to examine both normal and pathological cerebrospinal fluids for these factors.

Many methods of plasminogen assay have been described and they all depend on conversion to plasmin by an activator and estimation of proteolytic or esterolytic activity. We tried several of them but all were too insensitive for our purpose and we devised the following method to deal with the problem.

The high degree of sensitivity of the fibrin plate for activator assay suggested that fibrin was probably the best substrate to use, and our method involves measuring the lysis time of a standardized fibrin clot made from bovine fibrinogen. Plasminogen is supplied by the fluid being tested, streptokinase is added to the mixture, and the whole clotted with thrombin. The streptokinase activates the plasminogen and the fibrin is lysed. If all other factors remain constant, the lysis time depends on the amount of plasminogen in the test fluid.

The technique (Fig. 1) is as follows:

REAGENTS Bovine fibrinogen (Armour) 0·8% in buffer, phosphate buffer M/15 *p*H 7·4, thrombin (P.D. Topical), 50 u./ml. in saline, streptokinase (Wellcome), 100 u./ml., in saline.

METHOD Test tubes, 5 in. × ½ in., are used and each contains 1 ml. fibrinogen solution, 1 ml. buffer, 0·1 ml. streptokinase, 0·1 ml. cerebrospinal fluid, and 0·1 ml. thrombin.

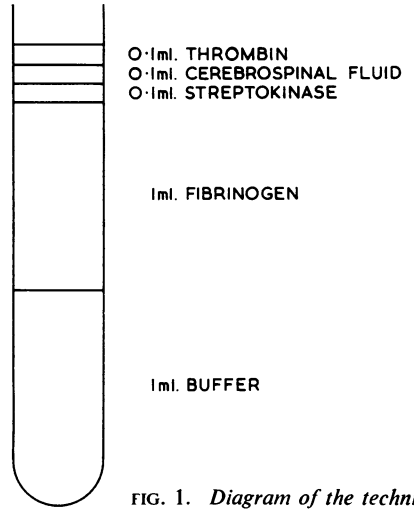

FIG. 1. *Diagram of the technique.*

Invert once to mix and avoid frothing. Place in a water bath at 37°C. as soon as clotting occurs.

We use a transparent water bath so that the clots can be seen without disturbing them, or an instrument of my own invention which measures lysis time photoelectrically.

The following technical points must be mentioned. Most batches of Armour fibrinogen seem to be satisfactory, but each must be checked against a known control, and the concentration of fibrinogen chosen must be high enough to give a firm clot with a reasonably short lysis time.

We found that 10 units of streptokinase is sufficient to give almost immediate activation of the amounts of plasminogen likely to be present without waste.

It is unnecessary to use plasminogen-free fibrinogen as the bovine plasminogen is not activated by streptokinase and the clots with no added plasminogen are stable for at least 48 hours in its presence.

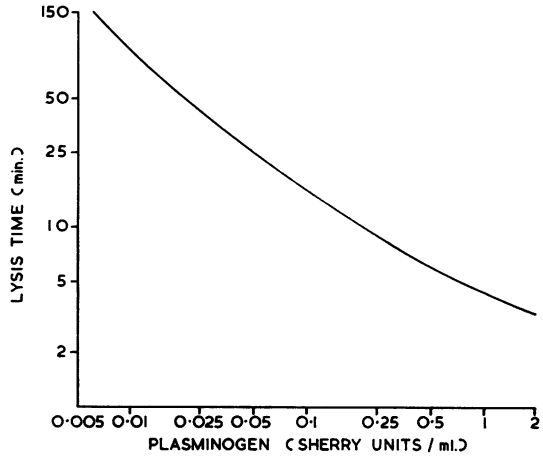

FIG. 2. *Relationship between plasminogen concentration and lysis time in a standardized system.*

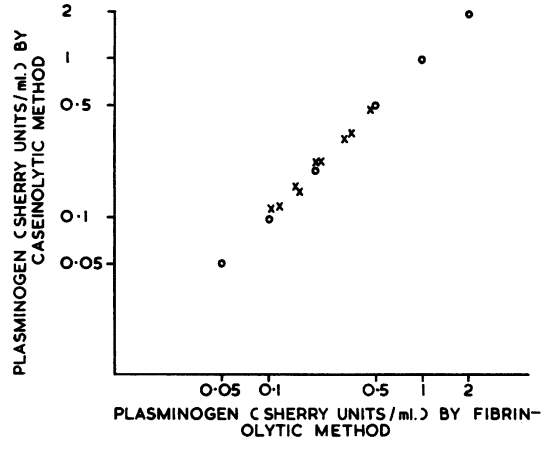

FIG. 3. *Comparison between results of plasminogen assays by caseinolytic and fibrinolytic methods.*

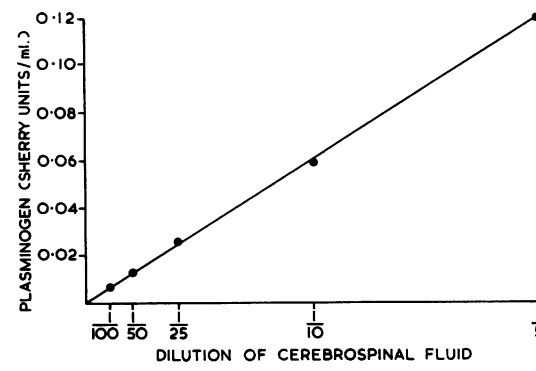

FIG. 4. *Effect of dilution of test specimen on plasminogen assay.*

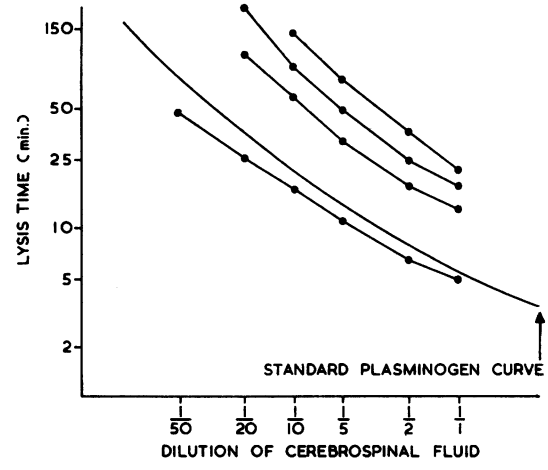

FIG. 5. *Effect of dilution of cerebrospinal fluid on plasminogen assay.*

With a source of plasminogen added, lysis times vary from a few minutes to several hours, enabling as little as 0·001 Sherry units of plasminogen to be detected.

A reference curve is drawn using known amounts of purified human plasminogen and lysis times are plotted against plasminogen units (Fig. 2). The end-point of lysis is quite sharp and results are reproducible.

In any system using biological fluids, unknown factors may be present, and it occurred to us that factors other than plasminogen might be affecting the lysis time. To exclude this possibility, results were checked by a modified caseinolytic method and good correlation was obtained (Fig. 3). Some of our specimens gave lysis times so short that accurate readings were impossible and the effect of dilution was investigated. We found that the test specimen could be diluted as necessary without affecting the result (Figs. 4 and 5).

The method may be modified in several ways. It can be used for streptokinase assay by using known quantities of human plasminogen but other factors must be absent or known. It can also be used to detect plasmin and distinguish it from plasminogen by adding ϵ-amino caproic acid (E.A.C.A.) to the system. Lysis is delayed for at least 24 hours in this system by adding E.A.C.A. at a final concentration of 10^{-3} M, at which concentration plasmin activity is also reduced by about 25 times. At a final concentration of 5×10^{-4}M E.A.C.A., 90% inhibition of activator is obtained but plasmin activity is reduced

by only about five times, thus giving adequate sensitivity to detect pre-formed plasmin.

Anti-plasmin may be demonstrated by differences in lysis times before and after treating the specimen with N/6 HCl and subsequent neutralization.

The method has several advantages over others. It is very sensitive. It is easily carried out and requires no special apparatus or reagents. It can be set up at short notice and results are quickly obtained. It appears to be suitable for routine use in the control of fibrinolytic therapy of the ventricular and subarachnoid cavities.

The instrument devised for measuring lysis times in this method operates on a photoelectric principle and incorporates a counting circuit which gives readings of lysis times in minutes. Once the test is set up the machine may be left to operate and the result can be read from the dial at any time.

DR. MCNICOL asked if antibody to streptokinase was present in the cerebrospinal fluid, because this would in fact produce an alteration in the activator content of the system and introduce a variable which would make the results impossible to interpret.

DR. NEWMAN replied that he had been unable to find any evidence of anti-activator in any of the specimens he had examined.