FIG. 2. Comparison of the results of testing 79 C-reactive protein-positive sera by the capillary precipitin and gel-diffusion precipitin methods.

method has been compared with the conventional capillary precipitin method and with a latex slide test and has been found to be intermediate in sensitivity.

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A method for the comparison of the antiplasmin activity of two groups of blood sera

MARGARET HOWELL *From the Department of Haematology, St. George's Hospital, London*

PRINCIPLE

The presence of antiplasmin activity in a blood sample can be shown by its ability to prolong the lysis time of a plasmin-enriched standardized fibrin clot, the extent of prolongation varying directly with the level of antiplasmin activity. Plasmin in such experiments is usually formed by the autoactivation of pure plasminogen, so that it is free from both activator and antiplasmin activity. Pure plasminogen is difficult to prepare and when obtained commercially is both expensive and unstable, so the following method has been devised for the measurement of antiplasmin activity using materials readily obtainable in any clinical laboratory.

Plasmin is produced by the streptokinase activation of the euglobulin fraction of normal serum. All serum plasminogen is precipitated in this fraction and the antiplasmins remain in the supernatant (Kowalski, Kopeč, and Niewiarowski, 1959; Flute, 1960). Epsilon-amino-caproic acid (E.A.C.A.) is added after activation to neutralize any remaining activator activity. As a source of fibrinogen for the clot, human plasma euglobulin is used. The clot is arranged to contain E.A.C.A. at a concentration of 10^{-3} molar. At this level it will inhibit further streptokinase activation of plasminogen contained in the plasma euglobulin without affecting the plasmin proteolysis of fibrin (Alkjaersig, Fletcher, and Sherry, 1959).

The actual tests are best carried out on serum, as the differing fibrinogen content of plasma samples may influence lysis times.

MATERIALS

- 1 PHOSPHATE BUFFER pH 7.4 Na_2HPO_4 , 9.47 g., is dissolved in 1 litre of distilled water. To this is added 3.02 g. KH_2PO_4 dissolved in 250 ml. distilled water.
- 2 BOVINE THROMBIN (Leo Pharmaceutical Products) Dissolved in buffer to 50 N.I.H. units/ml.
- 3 STREPTOKINASE (Varidase, Lederle Laboratories) Dissolved in buffer, stable for at least one month when stored in aliquots at -30°C .
- 4 EPSILON-AMINO-CAPROIC ACID (Light and Co.) A 10^{-2} molar solution in buffer.

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5 SERUM EUGLOBULIN This is prepared from pooled human serum as follows: One part of serum is added to 18 parts of distilled water and the pH adjusted to 5.3 by the addition of 0.2 parts of 1% acetic acid. This is left at approximately 4°C. for 30 minutes to allow the euglobulin fraction to precipitate. It is then centrifuged at 1,500 r.p.m. (700 *g*) for 15 minutes and the supernatant discarded. The precipitate is washed once with distilled water at pH 5.3 and then redissolved in buffer to one-fifth of the original serum volume. Stored in aliquots at -30°C. it is stable for at least three months.

6 PLASMA EUGLOBULIN This is prepared from pooled time-expired A.C.D. plasma in the same way as the serum euglobulin, and redissolved in buffer to its original plasma volume. This is also stored at -30°C.

METHOD

PREPARATION OF PLASMIN One part of streptokinase solution and 8 parts of serum euglobulin solution are incubated together at room temperature for five to 10 minutes. Then 1 part of E.A.C.A. solution is added. The plasmin produced is stable, if kept in melting ice, for at least five hours.

PREPARATION OF 'FIBRINOGEN' FOR THE CLOT To 2 parts of plasma euglobulin 4 parts of buffer and 1 part of E.A.C.A. are added.

A clot is made as follows:

Plasmin 0.2 ml.
 'Fibrinogen' 0.7 ml.
 Buffer 0.05 ml.
 Thrombin 0.1 ml.

The time between the addition of thrombin and lysis of the clot is taken as clot lysis time. A small thread of material which sometimes remains after lysis is complete may be ignored. The concentration of streptokinase used for preparation of the plasmin is adjusted until the clot lysis time is about 10 minutes. This concentration is constant for each batch of streptokinase, provided that the same batches of serum and plasma euglobulin are used.

DEMONSTRATION OF ANTIPLASMIN ACTIVITY Test serum, 0.05 ml., is added to 0.2 ml. plasmin and incubated at

room temperature for 15 minutes. Then 0.7 ml. fibrinogen is added and the mixture clotted with 0.1 ml. thrombin, a stop watch being started immediately. The tubes are placed in a 37°C. water-bath and the lysis time recorded.

RESULTS

These may be recorded as prolongation of clot lysis time in minutes, or a dilution curve may be made using pooled normal serum and the results expressed as percentage antiplasmin activity. The curve is made as follows:—

Tube No.	% Serum (Antiplasmin Activity)	Serum (ml.)	Buffer (ml.)
1	100	0.2	—
2	75	0.3	0.1
3	50	0.2	0.2
4	25	0.1	0.3
5	0	—	0.2

From each dilution 0.05 ml. is incubated with 0.2 ml. of plasmin for 15 minutes before the addition of 'fibrinogen' and thrombin. The percentage activity is then plotted against lysis time in minutes, and the activity of the test sera read off the resulting curve.

SUMMARY

A clot lysis method for estimating serum antiplasmin activity is described using streptokinase-activated serum euglobulin as a source of plasmin, and human plasma euglobulin as a source of fibrinogen. Epsilon-aminocaproic acid is used to prevent residual activator activity in the clot.

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