Rapid Method for Determination of Plasma Fibrinogen.*† (25427)

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Since determination of fibrinogen is most useful in hemorrhagic emergencies, the present fairly lengthy methods constitute a definite drawback to its routine use. A technic to determine the level of plasma fibrinogen rapidly and without excessive loss of accuracy would be of obvious value. The procedure described here seems to fulfill these requirements.

Principle of method. Defibrinated plasma does not clot upon addition of thromboplastin and calcium or of thrombin. When, however, a source of fibrinogen is added to defibrinated plasma, clotting time upon addition of thromboplastin and calcium is inversely proportional to concentration of fibrinogen in mixture. *Materials*: (a) *Defibrinated plasma* (substrate reagent). Nine volumes of bovine or of human blood are collected in 1 volume of 0.1 M sodium citrate. Plasma is separated by centrifugation at 2,000 rpm/5 min. Equal volumes of bovine and human plasma are mixed. For defibrination, 5 N.I.H. units of bovine thrombin (Parke & Davis) in 0.1 ml of saline solution are added to 1 ml of plasma mixture. The clotted plasma is left at room temperature for 30 min. and the clot then separated by filtration through gauze. One unit of thrombin in 0.3 ml is again added. The new clot is also removed, the procedure being repeated twice. Completely defibrinated plasma contains optimum concentration of prothrombin and accessory factors. The defibrinated plasma is incubated at room temperature 2 hrs to allow full inactivation of thrombin. When then kept at −20°C in corked test tubes, the substrate reagent is stable and may be used satisfactorily for at least 8 months. It may also be lyophilized and kept at 4°C indefinitely, being reconstituted to original volume of plasma with distilled water at time of use. (b) *Rabbit brain thromboplastin* (Difco) or Permaplastin (C. W. Alban Co.); (c) $CaCl_2$ 0.03 M; (d) *Plasma to be tested*: Nine volumes of blood are collected in 1 volume of 0.1 M sodium oxalate. The plasma is separated by centrifugation at 3,000 rpm/5 min in angle centrifuge. Plasma is absorbed with 50 mg of barium sulfate for 5 min and centrifuged at 3,000 rpm/5 min. Dilutions of the supernatant plasma are prepared with imidazole buffer (pH 7.25). Defibrinated plasma, plasma to be tested diluted 1/5, thromboplastin and $CaCl_2$ all in the volume 0.1 ml are added rapidly in test tube kept in water bath at 37°C and clotting time recorded. Clotting time of mixture is then translated into plasma fibrinogen percentages by means of a curve previously prepared (Fig. 1). Only clotting values should be considered between 20″ and 50″, as the method is most accurate in this range. Occasionally, when fibrinogen concentration is very low it may be necessary to use undiluted plasma or plasma diluted only 1/2.5; with fibrinogen concentration very high it may be necessary to dilute the test plasma to 1/10 or 1/20. In such instances, the percentage values obtained are corrected on the basis of the dilutions used. *Preparation of dilution curve*: Five normal human plasmas were pooled and adsorbed with 50 mg $BaSO_4$/ml as in the test. Supernatant plasma was diluted 1:5 with imidazole buffer and this reagent taken to contain 100% fibrinogen. The reagent was then diluted further to 80, 60, 50, 40, 30 and 20% in imidazole buffer. Values in 5 sets of determinations showed a maximum difference of 1.6 seconds, the higher deviation being noted for the lowest and highest dilutions (Fig. 1).

Reproducibility and standard error of procedure. When dilutions were used giving clotting times between 50″ and 20″, determin-

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ations on 3 normal plasmas and several pathological plasmas, using 15 determinations of each, gave a standard error of ± 1.9 sec. When clotting times of undiluted plasmas were longer than 60″, severe fibrinogenopenia was present. Such results are indicated as "no clot." Results have been checked against those with the standard "tyrosine method." Good correlation has been found in patients with normal, elevated or decreased plasma fibrinogen level and in severe hypofibrinogenemia (Table I).

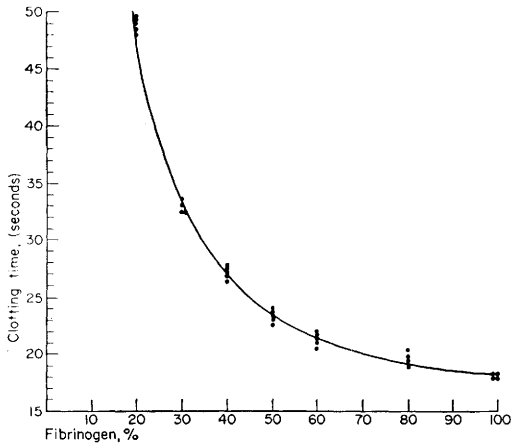

FIG. 1. Correlation of clotting times to plasma fibrinogen percentage values.*

* Clotting times are reported in sec. on ordinate; percentage levels of fibrinogen are reported on abscissa. Thus, clotting time values are readily translated into plasma fibrinogen percentages. The 5 points represent 5 actual experiments using the same system, with plasma pool where fibrinogen content was of 300 mg % by the tyrosine method. Continuous line is avg of the 5 values. As the normal plasma was originally diluted 1:5, the method can be used to study fibrinogen levels from 12 to 1200 mg %. No clot was obtained when plasma was diluted 1:25 or more.

TABLE I. Correlation of Present Method to Tyrosine Method.

No. of cases	Diagnosis	Tyrosine method, mg % (Avg values)	Present method, mg %* (Avg values)
1	Carcinoma of lung	430	388
2	Pulmonary infarction	380	360
2	Arterioselerosis	400	335
5	Myocardial infarction	292	305
3	Congestive heart failure	272	240
10	Mitral stenosis	368	332
2	Subacute bacterial endocarditis	285	302
2	Rheumatic heart disease	257	267
2	Intestinal obstruction	317	320
1	Fistula in ano	340	464
1	Perianal abscess	365	388
1	Infectious hepatitis	202	235
1	Cirrhosis of liver	100	104
3	Diabetes mellitus	224	220
1	Carcinoma of pancreas	214	168
2	Acute thrombophlebitis	330	360
1	Posthemorrhagic anemia	225	180
2	Fractures	425	447

* Values in mg % are calculated from curve of Fig. 1 where 100% value represented 300 mg % by the tyrosine method. Values were multiplied by 2 or 4 when plasma was diluted 1/10 or 1/20; or divided by 2 or 5 when plasma was diluted 1/2.5 or used undiluted.

Summary. A method is presented for rapid and, under certain conditions, accurate determination of plasma fibrinogen level. The method correlates well with standard tyrosine technic. This method should find its greatest use in rapid determination of plasma fibrinogen level in hemorrhagic catastrophes and especially in hemorrhagic accidents of pregnancy.

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Experimental Penicillin Prophylaxis of Staphylococcal Infection in Rabbits. (25428)

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The problem of *Staphylococcus aureus* infections in clean surgical wounds is receiving widespread attention. One method to control this is administration of penicillin. It is currently believed that, in addition to its undesirable side-effects, penicillin is of little or no

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