PREPARATION OF THROMBIN

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Potent preparations of thrombin have been described by Mellanby (1), Howell (2), Bleibtreu (3), and Seegers, Brinkhous, Smith, and Warner (4). The present paper describes a method which, according to our experience, is the most convenient for preparing great amounts of a very potent thrombin.

EXPERIMENTAL

Plasma—Ox blood is obtained at the slaughter-house in glass jars containing 15 cc. of a 20 per cent potassium oxalate solution per liter of blood. As soon as possible after arrival at the laboratory the blood is passed through a metal sieve and centrifuged. There is obtained 50 to 55 per cent (by volume) of plasma, which is used immediately for preparing thrombin.

Thrombokinase—Fresh, ground beef lung is extracted with 2 parts of 0.9 per cent NaCl solution for 4 to 5 hours with intermittent stirring. The mixture is then passed through a double layer of gauze and used as such.

Units—A thrombin unit is defined as the amount of active substance which will clot 1 cc. of oxalated plasma in 30 seconds at 37°. To 0.10 cc. of the thrombin solution is added 1.0 cc. of ice-cold oxalated plasma and the mixture is placed in a water bath at 37°. For clotting times not greater than 2 minutes the activity is directly proportional to the reciprocal of the clotting time.

Preparation of Crude Thrombin (A)—The following procedure was found to give the best results. To 1 liter of oxalated plasma are added 15 liters of cold distilled water and acetic acid until the pH is 5.3 (about 0.3 liter of a 1 per cent solution). After
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Standing for 5 to 10 minutes the mixture is centrifuged. In the meantime a new lot is precipitated, and this procedure is repeated until in about 1 or 2 hours 10 to 12 liters of plasma have been precipitated and passed through the centrifuge.

The precipitate corresponding to 1 liter of plasma is dissolved at 25–30° in 0.70 liter of a 0.9 per cent NaCl solution. Then 0.15 liter of thrombokinase and 25 cc. of a 1.5 per cent solution of calcium chloride are added (per liter of plasma). The mixture clots in 1 to 3 minutes and is stirred vigorously during this time. After it has stood for 1 hour, the fibrin is removed. The thrombin solution, which contains 6 to 8 thrombin units per cc., i.e. about 7000 units per liter of plasma, is then precipitated with 1 volume of acetone and, after standing overnight, is centrifuged. The precipitate is ground in a mortar with acetone and filtered on a Buchner funnel the next day. Again it is ground with acetone and after standing several days filtered and dried with ether. Yield 5.5 to 7 gm. per liter of plasma. About 1200 gm. of crude thrombin were prepared according to this method.

50 mg. of this crude thrombin give in 10 cc. of NaCl solution an activity of 3.5 to 4.0 thrombin units per cc., which corresponds to about 800 units per gm. of substance and 4800 units per liter of plasma; i.e., 70 per cent of the activity of the thrombin solution.

Purification of Crude Thrombin (B) 40 gm. of crude thrombin are extracted for 6 hours at 0° with 800 cc. of 0.9 per cent NaCl solution and a few drops of octyl alcohol. After centrifugation the precipitate is extracted again for 12 hours with 600 cc. of NaCl solution. Yield 1100 cc. of a solution containing 30 to 36 thrombin units per cc. 1 per cent acetic acid solution is added until the pH is 5.5. The precipitate is discarded after centrifugation and the solution is poured into 2 volumes of ice-cold acetone. After standing for 2 hours the mixture is centrifuged and the precipitate ground with acetone in a mortar. After further standing for 24 hours it is filtered, treated with absolute alcohol, washed, and dried with ether. Yield 2.5 gm. of purified thrombin containing about 10,000 units per gm., equal to about 80 per cent of the activity in the crude thrombin.

Properties of Purified Thrombin—Of the purified thrombin (B) 85 to 90 per cent is soluble in distilled water. Of such a solution
a curve for the precipitation with increasing amounts of ammonium sulfate was obtained according to Schmitz (5) (Fig. 1, Curve I). The curve shows characteristic peaks for globulin and albumin, and, by fractionation with ammonium sulfate two or three times, an albumin fraction could be obtained, which after dialysis contained nearly all the activity; the precipitation curve for this fraction is shown in Fig. 1, Curve II. The globulin frac-

![Fig. 1. Precipitation with ammonium sulfate. Curve I, solution of Thrombin B; Curve II, active albumin fraction; Curve III, inactive globulin fraction.](http://www.jbc.org)

tion was small and practically inactive and gave Curve III, Fig. 1. Precipitating the dialyzed albumin fraction with acetone destroyed about 25 per cent of the activity.

Thrombin thus seems to be connected with proteins of the albumin type. This is interesting in view of the possibility of prothrombin being of globulin character, as it is precipitated by half saturation with ammonium sulfate according to Cekada (6) and Schmitz (7). Work on its purification is in progress.
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SUMMARY

1. A convenient method for preparing great amounts of a potent thrombin preparation is described.
2. Thrombin seems to be an albumin.

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