pletion of serum opsonins. These results confirm that the rate of carbon blood clearance is a reliable method for measuring phagocytic activity of R.E. cells.

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Received February 6, 1963. P.S.E.B.M., 1963, v112.

ε-Aminocaproic Acid and Urinary Trypsin Inhibitor: Potentiated Inhibition of Urokinase Induced Fibrinolysis.* (28240)

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In a comparative study of some natural protease inhibitors acting upon the fibrinolytic system it was observed that ε-aminocaproic acid (EACA) potentiated the inhibition of the urinary plasminogen activator (urokinase) by the trypsin inhibitor present in human urine. Since this trypsin inhibitor is normally present in the organism, and because EACA is used in the control of hemorrhage caused by excessive fibrinolysis, this observation is of immediate interest in evaluating the effects of EACA in the body.

Materials. 1. ε-aminocaproic acid (reprecipitated from methanol with ether). Stock solution: 20 mg/ml in barbital buffer: 0.05 M; pH 7.75. Further dilutions with barbital-gelatine buffer.

2. Urine inhibitor (mingin) was isolated from human pregnancy urine(1) and purified by methods to be described elsewhere. 1.6 mg completely neutralized 1.0 mg crystalline, pure trypsin.† Solutions were prepared in barbital-gelatine buffer.

3. Urokinase.‡ 2.300 Ploug units/mg. Stock solution: 920 units/ml barbital-gelatine buffer.

4. Thrombin,‡ bovine. 90 NIH units/mg. 10 units/ml barbital-gelatine buffer.

5. Fibrinogen (bovine) prepared after(2) and reprecipitated with phosphate(3).

6. Barbital buffer: 0.05 M; pH 7.75, containing 0.1 M NaCl.

7. Barbital-gelatine buffer: As above with 0.25% gelatine to prevent adsorption to glass.

Method. Clot lysis was followed by means of the thrombelastograph(4,5). This method was used in order to approach physiological conditions in the comparative study. Final reaction mixture contained 0.285% fibrinogen, barbital buffer, appropriate concentrations of inhibitors, urokinase, and was clotted with 1.4 units of thrombin per ml. Clotting time: 1 min. Lysis time was recorded in mm (2 mm = 1 min) measured from the first to the last oscillation on the thrombelastogram.

Results. A reference curve was obtained with varying concentrations of urokinase. The data yielded a straight line in a double logarithmic diagram, Fig. 1 (reference curve for the following experiment). Other experiments showed linearity over a wider range justifying the extrapolation in Fig. 1.

EACA is a weak inhibitor of fibrinolysis and in low concentration even enhances fibrinolytic activity. When tested against urokinase (22 units/ml reaction mixture) it was found that 8.16 mM EACA was the high-

* Supported by grant from U. S. Public Health Service, Nat. Heart Inst.

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TABLE I. Inhibitory Effect of Mingin on Urokinase in Presence or Absence of Added EACA. Concentration of Urokinase in Final Solution: 22 units/ml. EACA: 8.16 mM. Mingin concentrations recorded in μg/ml final solution.

Mingin conc (μg)	Lysis time No EACA (mm)	Lysis time With EACA (mm)
120	14	incomplete
90	13	110
60	12	53
30	9.5	19
15	8.5	13
7.5	8.0	10
0	7.5	7.5

est concentration (final) which did not produce measurable inhibition. With mingin (without and with EACA) results as in Table I were obtained. EACA clearly potentiated the inhibition by mingin. After conversion into units of free, active urokinase by interpolation on the reference curve in Fig. 1, and calculation of the amount of urokinase bound, Fig. 2 could be drawn. It is seen that 120 μg mingin per ml alone neutralize 15.2 units of urokinase (Curve A). In the presence of EACA the same effect is produced by only 19 μg mingin per ml (Curve B) representing an apparent increase in potency of about 6 times.

A more striking picture of the same effect was obtained when the concentration of mingin was kept constant, while the concentration of EACA was varied, (Table II). Calculation of the bound urokinase (after interpolation on a reference curve similar to Fig. 1) produced Fig. 3. It is seen that EACA at concentrations at which it normally enhances the fibrinolytic activity of urokinase still potentiated the inhibitory effect of mingin.

TABLE II. Effect of EACA on Fibrinolytic Activity of Urokinase in Presence and Absence of a Constant Concentration of Mingin. Final concentration of urokinase: 22 units per ml. Mingin: 30 μg per ml. EACA concentration in final mixture as recorded.

EACA (mM)	Lysis time No mingin (mm)	Lysis time With mingin (mm)
17.8	11.5	30
14.5	9.0	24
10.9	8.0	23
7.25	7.0	19
5.42	6.0	17
3.51	4.5	14
0	7.5	9.0

Discussion. The observations demonstrate

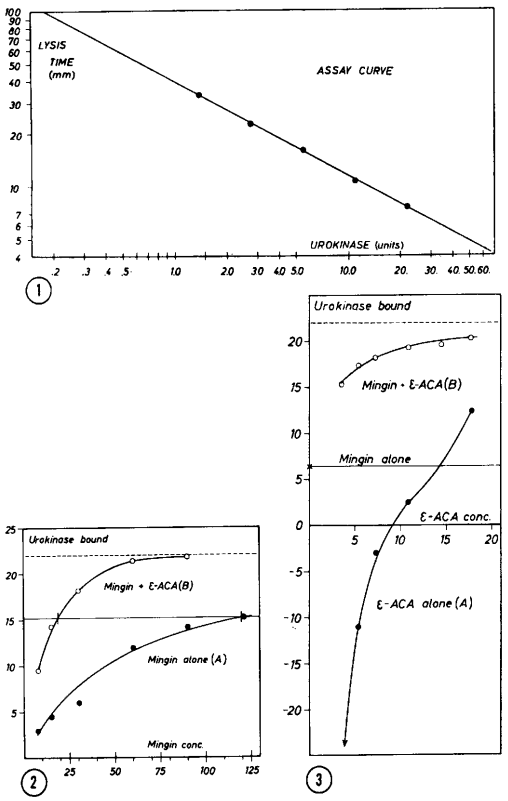

FIG. 1. Reference curve for urokinase. *Abscissa:* Concentration of urokinase in units per ml final reaction mixture (logarithmic). *Ordinate:* Lysis time read in mm on the thrombelastogram (2 mm = 1 min), (logarithmic).

FIG. 2. Neutralization of urokinase by mingin in presence and absence of EACA. *Abscissa:* Concentration of mingin in μg/ml final solution. *Ordinate:* Units of urokinase bound per ml final solution. Upper broken line indicates total amount of urokinase present in each experiment. Curve A: Urokinase neutralized by mingin alone. Curve B: Urokinase neutralized by mingin in presence of a constant concentration of EACA.

FIG. 3. Neutralization of urokinase by a constant concentration of mingin in presence of various concentrations of EACA. *Abscissa:* Final concentration of EACA in mM. *Ordinate:* Activity read as corresponding concentration of bound urokinase in units per ml. Upper broken line represents total concentration of urokinase in each experiment. Lower thin line represents bound urokinase when solutions contain only a constant amount of 30 μg mingin/ml. Curve A: EACA alone. Negative values represent the enhancement of fibrinolytic activity caused by small concentrations of EACA recorded as apparent increase in concentration of free urokinase. Curve B: EACA with constant concentration of mingin (30 μg/ml).

that a fibrinolytic agent (urokinase) and an inhibitor (mingin), both normally present in the human body, are greatly influenced in their effects by the presence of EACA. While higher concentrations of EACA inhibit the fibrinolytic activity of urokinase small concentrations enhance the effect. EACA greatly potentiates the inhibitory effect of mingin on urokinase. This was observed even at low concentrations where EACA normally enhances urokinase induced fibrinolysis.

The results obtained are of importance, because administration of EACA as an antifibrinolytic agent is gaining widespread use. The potentiating effect of even small amounts of EACA on inhibition of urokinase by mingin is especially significant, since this inhibitor is normally excreted in human urine(6). The excretion increases considerably during pregnancy. An average excretion per 24 hours of around 15,000 units (one inhibitor unit equals 1 μg trypsin) is reached during the third trimester followed by a steep fall after parturition(7). Large increases in excretion occur also after administration of ACTH or cortisone as well as during various forms of stress(8). The concentration of mingin per ml urine encountered in such cases is in the same range as used in the experiments described above. Hence, the potentiating effect of EACA on the inhibition by mingin should be taken into consideration when EACA is administered to patients.

Summary. The antifibrinolytic activity (assayed against urokinase) of the trypsin inhibitor (mingin) isolated from human urine is greatly potentiated by ϵ-aminocaproic acid. Potentiation of inhibition occurs even at concentrations of EACA where normally the fibrinolytic activity of urokinase is enhanced.

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Received February 15, 1963. P.S.E.B.M., 1963, v112.

Abnormalities in Chick Embryos Following Thalidomide and Other Insoluble Compounds in the Amniotic Cavity.* (28241)

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Thalidomide (N-pthaloyl glutamimide) has been implicated in a variety of congenital defects of infants whose mothers were given the drug during early pregnancy(1,2,3,4,5,6). The most common defects were amelia, phocomelia, defects of the digits and alimentary abnormalities. Other defects included hemangiomas of the nose and upper lip, dysplasia of the external ears or eyes and defects of the heart and genitourinary system. Since the chick embryo has been used in this laboratory to study the teratogenic activities of viruses(7,8,9,10) and antitumor drugs(11), it seemed that these techniques might be useful to study the teratogenic activity of thalidomide. Introduction of thalidomide into the amniotic cavity of early embryos resulted in encephalocele and eye or eyelid defects in a significant percentage of the treated embryos. However, these defects in no way resembled the thalidomide-induced defects of the human. The thalidomide was not readily soluble in the diluents used and some of it was observed to remain undissolved for several days in the amniotic cavity of the chick embryos. Further investigation showed that other insoluble compounds also produced similar abnormalities following amniotic inocu-

* Supported by USPHS grant from Nat. Inst. of Allergy and Infect. Dis.