promazine during cold exposure of hamsters (11).

The use of propiopromazine and chlorpromazine would, therefore, seem contraindicated in tests involving thermal stress. However, this study indicates that propiopromazine shows less interference with temperature regulation than chlorpromazine at a non-stressful environmental temperature.

These tranquilizers could be of benefit as an adjunct to the induction of hypo- or hyperthermia in animals without the artifacts introduced by emotional excitation or general anesthesia.

*Summary.* Four young adult mongrel dogs were exposed twice untranquilized to each of 3 environmental temperatures: 4.4°C, 23.9°C and 37.8°C and exposed twice tranquilized with 2.2 mg/kg propiopromazine hydrochloride. Rectal temperatures were monitored and recorded continuously during 2-hour exposures. Little difference was noted in rectal temperature response for tranquilized and untranquilized animals at the 23.9°C exposure. Tranquilized animals showed a greater decline in internal temperature at an environmental temperature of 4.4°C than control and when tranquilized showed a rise in rectal temperature during heat exposure (37.8°C) while control animals showed a decline. These results indicate an impairment in both heat loss and heat conservation mechanisms in the tranquilized animals during thermal stress, but little, if any alteration of temperature control at a non-stressful ambient temperature.

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## Anti-Fibrinolytic Effect of ε-Aminocaproic Acid as Measured by *in vivo* Clot Lysis.* (29106)

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ε-Aminocaproic acid (EACA) was first described as *in vitro* fibrinolytic inhibitor in a British patent issued to a Japanese firm in 1957(1). The anti-fibrinolytic action has been confirmed by other investigators(2,3). *In vitro* studies by Alkjaersig *et al*(4) and by Troll and Sherry(5) have shown that plasminogen activation includes a proteolytic step in which bonds between lysine and other amino acids are split, and that lysine and ornithine both act as inhibitors of this activation. In view of the close structural resemblance of EACA to these compounds, Sherry and his associates(2) suggested that the main inhibitory action of EACA is on

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plasminogen activation and not on plasmin *per se*. Further studies have shown that EACA also exerts a secondary inhibitory action on plasmin at concentrations greater than those necessary to inhibit plasminogen activation(6).

Lang and Bitz(7) have shown that EACA is without effect on the growth rate of rats when they are fed a diet containing 100 mg of EACA daily over a 7-week period, with the major portion of the material being excreted unchanged in the urine. EACA has been tested in a variety of clinical and experimental situations(2,8,9,10) in which it has been found to be capable of temporarily abolishing some pathological fibrinolysis. More recently Bale and Spar(11) have studied the effects of 2% EACA in drinking water consumed by rats bearing either the Murphy-Sturm lymphosarcoma or the Walker carcinoma 256. In their studies EACA increased several-fold the tumor localization of $I^{131}$-labeled fibrinogen given intravenously.

The experiments reported here were designed to investigate *in vivo* anti-fibrinolytic properties of EACA in the rat. Radioiodinated rat fibrinogen and radioiodinated antibody to rat fibrinogen were used as tracers to determine the extent of fibrinolytic inhibition. The test system involved the use of subcutaneous rat plasma clots containing either $I^{131}$-fibrinogen, $I^{131}$-antibody, or in one case, $I^{131}$-γ-globulin. Daily whole-body radioactivity measurements after clot production provided a simple but accurate means for determining the effectiveness of various orally administered doses of EACA on the gross retention of labeled materials.

*Materials and methods.* Sprague-Dawley female rats weighing 100-150 g were used. All animals were routinely maintained on drinking water containing potassium iodide ($6.25 \times 10^{-4}$ M) to reduce thyroid uptake of inorganic $I^{131}$. Crystalline EACA (Aldrich Chemical Co., Milwaukee, Wis.), in the concentrations indicated, was dissolved in drinking water.

*Rat fibrinogen.* Cohn's fraction I(12) was prepared from oxalated rat plasma and from this, fibrinogen was isolated by the method of Laki(13). The final product, dissolved in pH 8.0 borate buffer(14), contained 8-10 mg of protein per ml as measured by the Folin-Phenol reagent method of Lowry(15). Of this 90-95% was clottable with thrombin (bovine thrombin—Upjohn Co.) as determined by micro-Kjeldahl analysis.

*Antibody to rat fibrinogen.* Antibody to rat fibrinogen was purified from the sera of rabbits previously immunized with rat fibrinogen according to a scheme developed by Bale *et al*(16). Antisera were mixed with citrated rat plasma to complex anti-fibrinogen antibody with fibrinogen. Clotting was induced by addition of thrombin plus calcium to convert the antibody-fibrinogen complex to an insoluble antibody-fibrin complex. The fibrin-bound antibody was eluted with phosphate buffer (pH 11.6) which ruptures bonds between insoluble antigen (fibrin) and antibody without denaturation. After iodination, this product was repurified as described above.

*Rat γ-globulin.* Rat γ-globulin was prepared from rat serum by ammonium sulfate precipitation according to the method of Jager and Nickerson(17).

*Labeling of proteins with $I^{131}$.* Proteins were labeled with Oak Ridge $I^{131}$ using the iodine monochloride protein iodination method of Helmkamp *et al*(18) as further modified by Bale *et al*(19). The final labeled products contained an average of 2-3 atoms of I per protein molecule as calculated from the molar ratio of iodine monochloride to protein.

*Preparation of labeled clotting mixture for subcutaneous injections.* Clottable mixtures were prepared for injection by adding tracer amounts of labeled protein to a mixture of equal volumes of citrated rat plasma and 0.85% sodium chloride. The mixture was chilled in an ice-bath and, to induce clotting, one-tenth the plasma volume of 2% calcium chloride was added immediately prior to injection. These mixtures remained fluid for periods of at least 30 minutes after addition of calcium when kept in the ice-bath, but formed a firm clot within 1-2 minutes after being warmed to body temperature. Rats were anesthetized with a mixture of carbon dioxide and oxygen (1 to 1 by volume) and

0.75 ml of the cold, liquid $I^{131}$-clotting mixture was injected subcutaneously into the right mid flank area. The subcutaneous clots thus formed are referred to as $I^{131}$-fibrinogen, $I^{131}$-antibody or $I^{131}$-γ-globulin clots, depending on which of these materials was used as tracer in the clotting mixture.

*Radioactivity measurements.* Whole-body retention of $I^{131}$ radioactivity was taken as a gross maximal measure of clot retention and was measured by immobilizing the rat in a 1-liter beaker. The beaker was positioned in a reproducible manner on a brass platform above a sodium iodide scintillation crystal. After correction for background radiation, whole-body counts were compared to those of an injection standard (a rat sacrificed immediately after injection with the clotting mixture, and stored frozen between daily counts). The "percentage whole-body retention" on any given day after clot injection was then calculated as follows:

$$\text{percentage whole-body retention on day X} = 100 \left( \frac{\frac{\text{whole-body counts on day X}}{\text{standard counts on day X}}}{\frac{\text{whole-body counts at injection}}{\text{standard counts at injection}}} \right)$$

*Results. Effect of EACA dose level.* Fig. 1 shows the effect of various dose levels of orally administered EACA on retention of clot-implanted $I^{131}$-fibrinogen at various times after injection. From these data it can be seen that both 2% and 5% EACA in drinking water enhance whole-body retention of clot-$I^{131}$-fibrinogen as compared to that retained in non-EACA treated rats (on Day 7 rats on 5% EACA retain 70.2% ± 2.8% of the injected $I^{131}$ label, rats on 2% EACA retain 44.4% ± 12.0%, and control rats retain 9.0% ± 6.5%). Furthermore, the data indicate that 5% EACA is probably more effective in inhibiting degradation of clot-implanted $I^{131}$-fibrinogen than is 2% EACA, although the difference is not statistically significant until Day 7.

Fig. 2 shows the results from a similar study in which the effect of various levels of EACA treatment on retention of $I^{131}$-antibody clots was examined. Again both 2% and 5% EACA are shown to be effective in inhibiting clot lysis, with the higher dose level of EACA being the more effective level.

A comparison of Fig. 1 and 2 shows that, in control rats (no EACA treatment), $I^{131}$-fibrinogen radioactivity is lost more rapidly than is radioactivity coupled to antibody. Obviously EACA (both 2 and 5%) prolongs the retention of both $I^{131}$-fibrinogen and $I^{131}$-antibody. Although on the basis of the first 4 days observations EACA appears to spare $I^{131}$-fibrinogen to a greater extent than $I^{131}$-antibody, the data do not permit a clear cut distinction to be made.

*Effect of pretreatment with EACA.* Studies were designed to ascertain whether or not the effectiveness of EACA treatment in enhancing retention of either labeled fibrinogen or antibody could be further increased by predosing rats with EACA prior to clot injection. Rats were predosed with either 2% or 5% EACA for 7 days prior to clot production, and were maintained on this same level of EACA for 7 days after clot production while whole-body retention of $I^{131}$ was measured daily. Control animals did not get EACA until the time of clot production. The results shown in Fig. 3 indicate that predosing with 5% EACA does not further enhance the effectiveness of EACA in inhibiting the loss of radioactivity from $I^{131}$-antibody clots. Identical findings were obtained when rats were predosed with 2% EACA prior to antibody clot production, and when rats were predosed with either 2% or 5% EACA prior to $I^{131}$-fibrinogen clot production.

*Studies on duration of the EACA-induced anti-fibrinolytic state.* Results from predosing experiments suggested that the anti-fibrinolytic effect of EACA is manifest very soon after initiation of EACA treatment and probably is dependent upon a continuous supply of EACA for its maintenance. To test this hypothesis more fully, the duration of the anti-fibrinolytic state was measured following cessation of EACA treatment. Conversely, the time required for establishment of an anti-fibrinolytic state was determined in animals not given EACA until several days after clot injection. The results depicted in Fig.

4 were obtained from rats injected with $I^{131}$-fibrinogen clots and treated with 5% EACA. Three days later, when an anti-fibrinolytic state was well established, EACA treatment of half of these rats was discontinued and whole-body retention of $I^{131}$ decreased grossly within 24 hours after withdrawal of EACA. Fig. 5 shows results from the converse experiment, in which rats bearing $I^{131}$-fibrinogen clots were not given 5% EACA until 3 days after clot injection. Although the difference is not as striking, EACA promotes retention of radioactivity which becomes statistically significant 4 days after being started. Similar experiments performed with rats bearing $I^{131}$-antibody clots revealed qualitatively similar results.

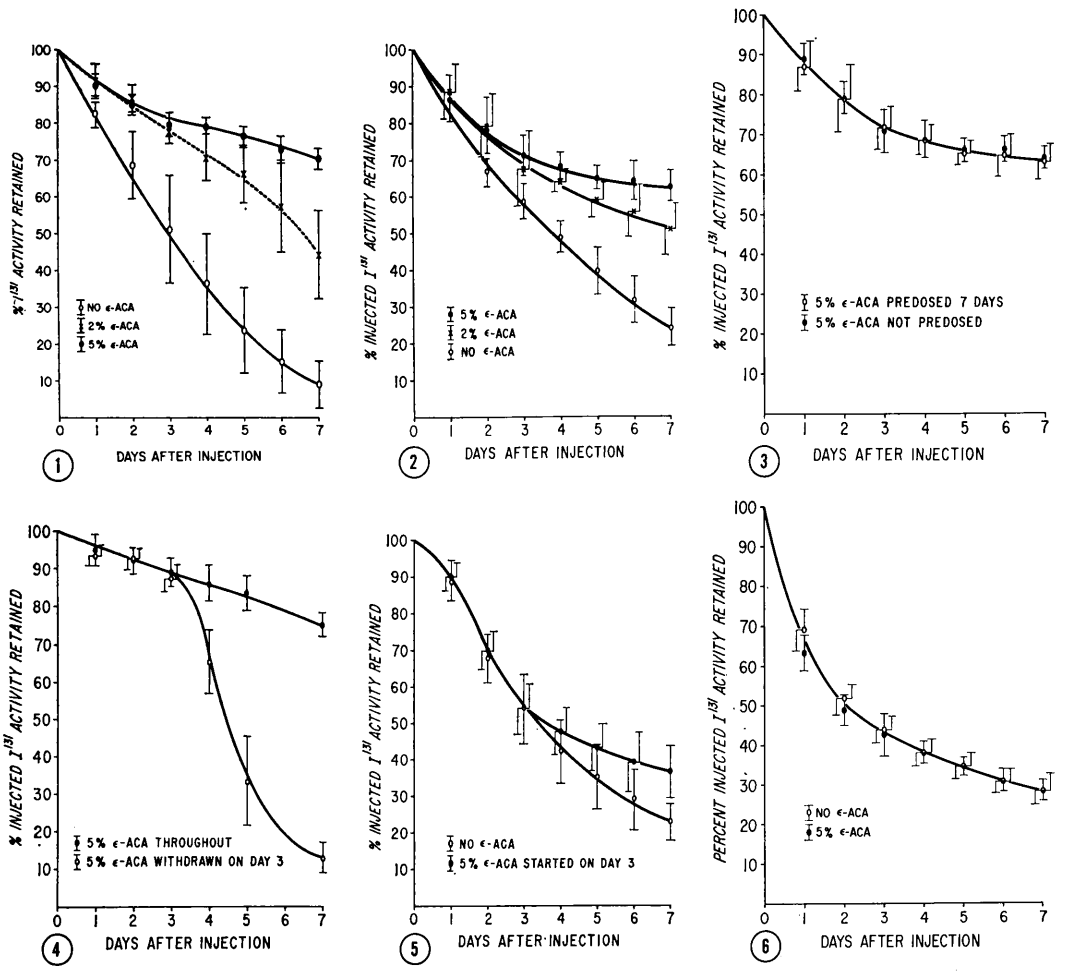


FIG. 1. Effect of various dosage levels of EACA on whole-body retention of $I^{131}$-fibrinogen clots (6 $\mu$c $I^{131}$/clot). Values plotted are average values from groups of 6 rats with corresponding standard deviations.

FIG. 2. Effect of various dosage levels of EACA on whole-body retention of $I^{131}$-antibody clots (16 $\mu$c/clot). Each point represents average value from a group of 10 rats.

FIG. 3. Effect of predosing rats for 7 days with 5% EACA prior to $I^{131}$-antibody clot injection (16 $\mu$c/clot). Each point represents average value from a group of 10 rats.

FIG. 4. Data showing the rapidity with which EACA-induced anti-fibrinolytic effect is destroyed following withdrawal of EACA treatment. (10 rats per group, 15 $\mu$c/clot.)

FIG. 5. Data showing time required for establishment of an EACA-induced anti-fibrinolytic state in rats with $I^{131}$-fibrinogen clots (15 $\mu$c/clot, 10 rats per group).

FIG. 6. Effect of treatment with 5% EACA on whole-body retention of $I^{131}$-$\gamma$-globulin clots (12 rats per group, 15 $\mu$c/clot).

*Lack of demonstrable effect of EACA on retention of clot implanted $I^{131}$-γ-globulin.* Although the results of the preceding experiments clearly demonstrate the effectiveness of orally administered EACA as an *in vivo* inhibitor of fibrinolysis, they shed no light on the question of the effect of this compound on plasma proteins not directly involved in blood coagulation. Rats were injected subcutaneously with a clotting mixture containing $I^{131}$-γ-globulin. Fig. 6 reveals that EACA has no effect on whole-body retention of $I^{131}$-γ-globulin included in a fibrin clot. These results strongly suggest that EACA acts primarily as an inhibitor of fibrinolysis and is not generally antiproteolytic.

*Discussion.* Results obtained during these studies are consistent with the hypothesis that ε-aminocaproic acid (EACA) is a potent *in vivo,* as well as *in vitro,* fibrinolytic inhibitor. Oral administration of EACA is shown to inhibit the disappearance of subcutaneous clots derived from $I^{131}$-rat fibrinogen and similarly labeled antibody against rat fibrinogen. Treatment with 5% EACA is shown to be more effective in this respect than is treatment with 2% EACA. Predosing with EACA prior to clot production does not produce a more heightened state of anti-fibrinolysis.

On the basis of results obtained from experiments in which treatment with EACA was either discontinued or initiated some 3 days after labeled clot production, it is concluded that the EACA-induced anti-fibrinolytic state is initiated promptly after administration of EACA. Administration of EACA must be continued to prevent equally prompt restoration of normal fibrinolysis.

Lang and Bitz(7) and McNicol(20) have shown that EACA is rapidly absorbed from the gastrointestinal tract following oral administration, with peak plasma levels attained in about 2 hours. Urinary excretion is rapid, 80 to 90% of a given dose being recovered unchanged in the urine. It would appear that the extent of inhibition of fibrinolysis within the subcutaneous clot, produced by orally administered EACA, is referable to the various dosage levels of EACA and probably reflects variations in the amount of EACA able to enter the clot area.

Sherry and co-workers(21) have presented a scheme for the physiological mechanisms of fibrinolysis in which it is stated that thrombi contain relatively high levels of plasminogen because of the affinity of this material for fibrin. It is thus reasonable to assume that the clots produced in our studies may be rich in plasminogen which is in turn normally readily convertible to active fibrinolysin (plasmin).

There are little or no published data on the role of plasminogen (plasmin) in normal animals. These studies strongly suggest that in normal animals plasmin can significantly facilitate removal of extravascular (subcutaneous) as well as intravascular fibrin.

That EACA acts solely as an inhibitor of fibrinolysis in a restricted sense, and not as a general proteolytic inhibitor, is borne out by results from studies with $I^{131}$-γ-globulin clots. In the latter case EACA treatment was without effect in inhibiting loss of the $I^{131}$ label. Furthermore a comparison of the effect of EACA on loss of activity from $I^{131}$-fibrinogen and $I^{131}$-antibody clots, suggests that EACA is somewhat more effective in inhibiting loss of $I^{131}$ from fibrinogen labeled clots. Accordingly one may speculate that EACA is only indirectly responsible for the observed inhibition of loss of $I^{131}$-antibody, and that EACA inhibits the degradation of antibody secondarily to inhibition of lysis of fibrin to which antibody is coupled. It is also conceivable that antibody to fibrinogen in some way protects fibrin from the lytic effects of plasmin.

*Summary.* The *in vivo* anti-fibrinolytic properties of ε-aminocaproic acid (EACA) were measured in rats by studying the effect of orally administered EACA on retention of subcutaneous rat plasma clots labeled with either $I^{131}$-fibrinogen or $I^{131}$-antibody to fibrinogen. Five percent EACA in drinking water was found to be a better fibrinolytic inhibitor than 2% EACA; predosing with EACA did not further enhance fibrinolytic inhibition; and the EACA-induced anti-fibrinolytic state was shown to be initiated and terminated within hours after initiation and

cessation, respectively, of EACA treatment. Results obtained support the hypothesis that EACA is a potent fibrinolytic inhibitor *in vivo*, as well as *in vitro*.

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## Effect of ε-Aminocaproic Acid on Deposition of Radioiodinated Fibrinogen and Antibodies to Fibrinogen in Turpentine-Induced Abscesses of the Rat.* (29107)

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Orally administered ε-aminocaproic acid (EACA) enhances the uptake of radioiodinated fibrinogen in some transplantable rat tumors(1). More recently oral administration of EACA has been shown to enhance whole-body retention of clot-implanted $I^{131}$-labeled fibrinogen and $I^{131}$-labeled antibody to fibrinogen in rats(2). The present report describes experiments in which was measured the effect of orally administered EACA on deposition and retention of intravenously injected $I^{131}$-rat fibrinogen and $I^{131}$-antibody to rat fibrinogen in subcutaneous turpentine-induced abscesses of the rat. Whole-body, abscess, and blood levels of $I^{131}$ were measured at 24-hour intervals over a 7-day period following injection. The effect of EACA treatment on whole-body and blood retention of these labeled proteins was also measured in normal rats.

*Materials and methods.* Sprague-Dawley female rats (100-150 g) were maintained on $6.25 \times 10^{-4}$ M potassium iodide drinking water to reduce thyroid uptake of inorganic $I^{131}$. Where used, EACA was administered in

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