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

1. British Patent Specification 770, 693. Her Majesty's Stationery Office, London, 1957.

2. Sherry, S., Fletcher, A. P., Alkjaersig, N., Sawyer, W. D., *Trans. Assn. Am. Physicians,* 1959, v72, 62.

3. Celander, D. R., Messer, D., Guest, M. M., *Texas Rep. Biol. Med.,* 1961, v19, 16.

4. Alkjaersig, N., Fletcher, A. P., Sherry, S., *J. Biol. Chem.,* 1958, v233, 86.

5. Troll, W., Sherry, S., *ibid.,* 1955, v213, 881.

6. Alkjaersig, N., Fletcher, A. P., Sherry, S., *ibid.,* 1959, v234, 832.

7. Lang, K., Bitz, H., *Biochem. Z.,* 1953, v324, 495.

8. Abe, T., Sato, A., *Keio J. Med.,* 1959, v8, 219.

9. Sato, S., Ishibashi, Y., Endo, T., Watanabe, T., Nakajima, K., *ibid.,* 1959, v8, 267.

10. Nilsson, I. M., Sjoerdsma, A., Waldenström, J., *Lancet,* 1960, v1, 1322.

11. Bale, W. F., Spar, I. L., Goodland, R. L., Izzo, M. J., *Fed. Proc.,* 1961, v20, 58.

12. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., Taylor, N. L., *J. Am. Chem. Soc.,* 1946, v68, 459.

13. Laki, K., *Arch. Biochem. Biophys.,* 1951, v32, 317.

14. Pressman, D., Eisen, H. N., *J. Immunol.,* 1950, v64, 273.

15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. *J. Biol. Chem.,* 1951, v193, 265.

16. Bale, W. F., Spar, I. L., Goodland, R. L., *Cancer Research,* 1960, v20, 1488.

17. Jager, B. V., Nickerson, M., *J. Biol. Chem.,* 1948, v173, 683.

18. Helmkamp, R. W., Goodland, R. L., Bale, W. F., Spar, I. L., Mutschler, L. E., *Cancer Research,* 1960, v20, 1495.

19. Bale, W. F., Helmkamp, R. W., Davis, T. P., Izzo, M. J., Goodland, R. L., Spar, I. L., Univ. of Rochester Atomic Energy Project Report, U.R. 604, 1962.

20. McNicol, G. P., Fletcher, A. P., Alkjaersig, N., Sherry, S., *J. Lab. Clin. Med.,* 1962, v59, 15.

21. Sherry, S., Fletcher, A. P., Alkjaersig, N., *Physiol. Rev.,* 1959, v39, 343.

Received September 26, 1963. P.S.E.B.M., 1964, v115.

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

LETITIA E. MUTSCHLER† (Introduced by W. F. Bale)

Department of Radiation and Biology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

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 6.25×10^{-4} M potassium iodide drinking water to reduce thyroid uptake of inorganic I^{131}. Where used, EACA was administered in

* This material was included in a Ph.D. thesis submitted to Dept. of Radiation Biology, Univ. of Rochester. This paper is based on work performed under contract with U. S. Atomic Energy Commission at Univ. of Rochester Atomic Energy Project, Rochester, N. Y.

† Present address: Biophysics Division, Nat. Inst. for Med. Research, The Ridgeway, Mill Hill, London, N.W. 7, England.

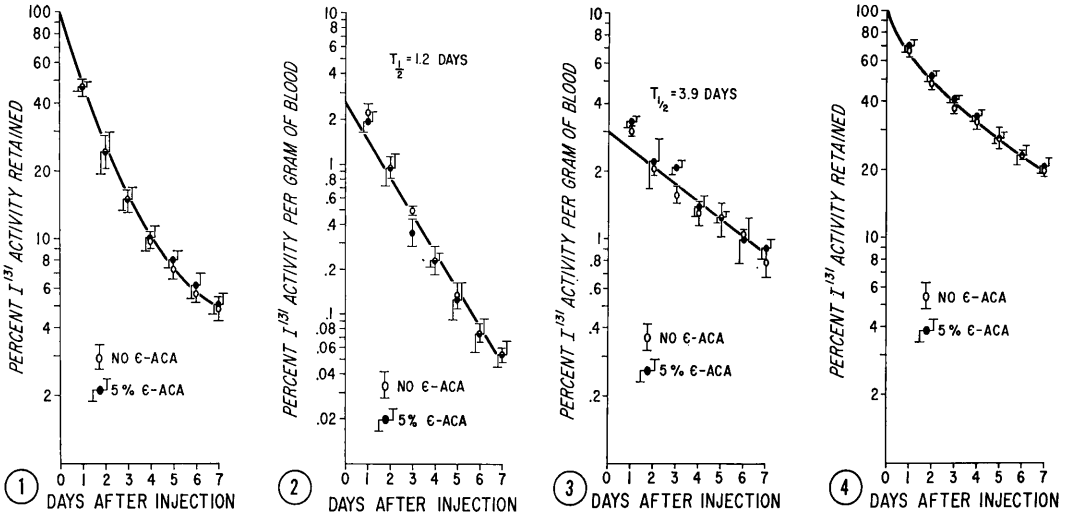


FIG. 1. Effect of 5% EACA on whole-body retention of I^{131}-fibrinogen in normal rats. Each rat received 0.25 ml of I^{131}-fibrinogen containing 23.4 μc of I^{131} via the saphenous vein. Values plotted are average values from groups of 5 rats with the corresponding group standard deviations.

FIG. 2. Effect of 5% EACA on blood levels of I^{131}-fibrinogen in normal rats (23.4 μc per rat, 5 rats per group).

FIG. 3. Effect of 5% EACA on blood levels of I^{131}-antibody in normal rats (24 μc per rat, 4 rats per group).

FIG. 4. Effect of 5% EACA on whole body retention of I^{131}-antibody in normal rats (24 μc per rat, 4 rats per group).

drinking water at a concentration of 5%.

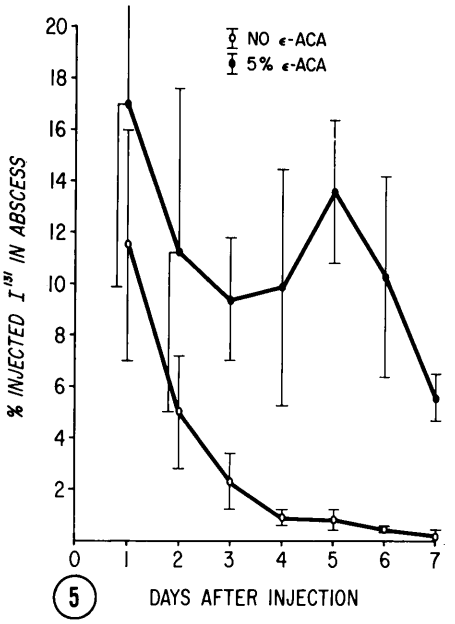
I^{131}-Rat fibrinogen and I^{131}-antibody to rat fibrinogen. These proteins were purified and labeled according to previously described techniques(2). For injection, labeled proteins were diluted with 0.85% sodium chloride solution to 100 μc I^{131} per ml and 0.25 ml was injected into the saphenous vein; during injection rats were anesthetized with carbon dioxide-oxygen (1 to 1 by volume).

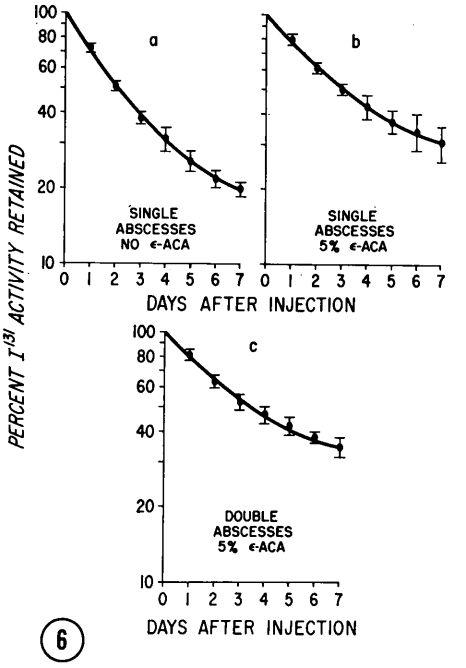
Subcutaneous turpentine-induced abscesses. Localized abscesses were produced by injecting 0.1 ml of turpentine subcutaneously. In experiments with single abscesses, turpentine was injected into the right mid-flank region; in experiments with double abscesses, abscesses were induced in both the right and left flanks. Labeled protein was given intravenously 2 hours after turpentine injection; pilot studies had shown that beyond 2 hours, abscess deposition of labeled protein decreased with increasing time between abscess implantation and protein injection.

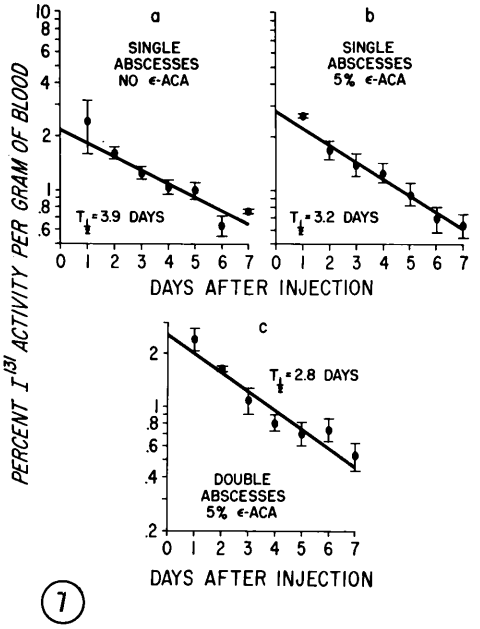
Radioactivity measurements. Whole-body retention of I^{131} was computed from daily whole-body counts. Blood half-life of labeled material was determined from radioactivity measurements made on samples obtained by cardiac puncture immediately prior to sacrifice. Abscesses were dissected after sacrifice and the amount of radioactivity therein measured. For each abscess the percentage of injected I^{131} and of retained whole body I^{131} was computed. In each experiment rats were sacrificed serially at 24-hour intervals over a 7-day period.

Results. Effect of EACA treatment on whole-body and blood retention of I^{131}-fibrinogen and I^{131}-antibody to fibrinogen in normal rats. The data presented in Figs. 1 and 2 show that 5% EACA has no effect on either whole-body or blood retention of I^{131}-fibrinogen in normal rats. As determined from Fig. 2 the blood half-life of I^{131}-fibrinogen is 1.2 days. Seven days after injection 5% of the injected I^{131} is retained (Fig. 1). Similarly, treatment of normal rats with 5% EACA had no effect on either blood half-life (Fig. 3) or whole-body retention (Fig. 4) of I^{131}-antibody to rat fibrinogen. In this case the blood half-life was 3.9 days and 20% of the I^{131}-label was retained 7 days after injection.

Studies in which rats bearing turpentine-







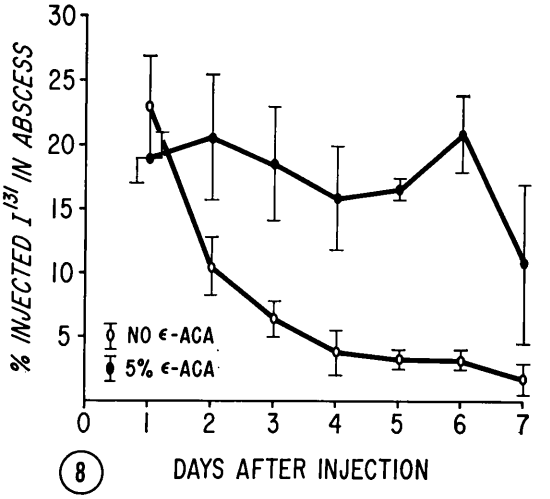


FIG. 5. Effect of EACA treatment on abscess accumulation of I^{131}-fibrinogen in single-abscess-bearing rats (30.8 μc per rat, 4 rats per group).

FIG. 6. Whole-body retention of I^{131}-antibody (31 μc per rat, 4 rats per group). (a) Single-abscess-bearing rats; no EACA. (b) Single-abscess-bearing rats; 5% EACA. (c) Double-abscess-bearing rats; 5% EACA.

FIG. 7. Blood levels of I^{131}-antibody (31 μc per rat, 4 rats per group). (a) Single-abscess-bearing rats; no EACA. (b) Single-abscess-bearing rats; 5% EACA. (c) Double-abscess-bearing rats; 5% EACA.

FIG. 8. Effect of EACA treatment on abscess accumulation of I^{131}-antibody in single-abscess-bearing rats (31 μc per rat, 4 rats per group).

induced abscesses were injected with I^{131}-fibrinogen. Treatment with 5% EACA had a pronounced effect on both whole-body and abscess retention, but not blood levels, of I^{131}-fibrinogen in rats bearing single abscesses. Seven days after injection, EACA treated rats retained twice as much I^{131} as did non-EACA treated rats (10% in the former, 5% in the latter). Fig. 5 depicts abscess accumulation of radioactivity in terms of the injected dose of I^{131} and clearly shows that EACA enhances abscess retention of I^{131}.

When rats were given double turpentine abscesses, injected with I^{131}-fibrinogen, and treated with EACA, whole-body retention of I^{131} was slightly increased over that in rats bearing single abscesses and treated with EACA. Again the blood half-life of the injected material was identical with that measured in normal rats. When total abscess (right plus left) content of I^{131} was measured, double-abscess-bearing rats showed a greater abscess content of radioactivity than did rats with single abscesses. However, while the radioactivity was equally distributed between the two abscesses, each abscess from a double abscess bearing rat did not contain as much I^{131} as did an abscess removed at the same time from a rat bearing only a single abscess.

Studies in which rats bearing turpentine-induced abscesses were injected with I^{131}-antibody to rat fibrinogen. Fig. 6 shows whole-body retention of I^{131}-antibody in single-abscess-bearing rats with and without EACA treatment and in double-abscess-bearing rats treated with EACA. These curves indicate that whole-body retention of labeled antibody in abscess-bearing rats, like that of labeled fibrinogen, is affected by EACA treatment. In contrast to the findings with I^{131}-fibrinogen, the blood half-life of I^{131}-antibody is also affected by EACA treatment in abscess-bearing rats. Blood levels of I^{131}-antibody, in abscess-bearing rats, are shown in Fig. 7. A comparison of Fig. 6 and 7 shows that while EACA treatment enhances whole-body retention of antibody label in rats with single abscesses, it decreases the amount of circulating antibody. The same effect is observed when a second abscess is introduced—whole-body retention of I^{131} increases, while circulating radioactivity and blood half-life decrease.

As shown in Fig. 8, abscess retention of I^{131}-antibody, in single-abscess-bearing rats, is also markedly increased by treatment with EACA. Results from double-abscess-bearing rats indicate that two abscesses retain more, but not twice as much, radioactivity than does an abscess removed from a single-abscess-bearing rat, and that the label is distributed evenly between the two abscesses.

Discussion. The results indicate that oral administration of 5% EACA has no effect on either whole-body retention or blood turnover of intravenously injected I^{131}-rat fibrinogen or I^{131}-antibody to rat fibrinogen in normal rats. As measured over the entire course of a 7-day period following labeled protein injection, labeled antibody ($T\ ½ = 3.9$ days) has a longer blood half-life than does labeled fibrinogen ($T\ ½ = 1.2$ days). The blood half-life of fibrinogen is unaffected by abscess implantation but whole-body retention is affected. Seven days after injection with labeled fibrinogen, 5% of the injected I^{131} is retained in normal rats (with or without EACA treatment); 5% is retained in rats with single abscesses and not treated with EACA; 9% is retained in single-abscess-bearing rats treated with EACA; and 12% is retained in double-abscess-bearing rats treated with EACA. These results suggest that fibrinogen localized in the abscess represents only a small fraction of the animal's fibrinogen-fibrin pool, with the blood stream being the major pool.

Studies in which abscess-bearing rats were injected with labeled antibody to rat fibrinogen show that abscess implantation affects not only whole-body retention of I^{131}, but also has a pronounced effect on the blood half-life of this protein. Whole-body retention of I^{131}-antibody increases in the same order as described for labeled fibrinogen, while blood half-life decreases in this order—3.9 days in normal rats (with or without EACA treatment); 3.9 days in rats with single abscesses (no EACA treatment); 3.2 days in rats with single abscesses treated with EACA; and 2.8 days in rats bearing double abscesses and treated with EACA.

Both labeled proteins localized in turpen-

tine-induced abscesses with labeled antibody showing a slightly greater abscess localization than labeled fibrinogen in non-EACA treated rats. Treatment with EACA enhances abscess retention of both proteins, the effect being more pronounced in the case of labeled fibrinogen. When two abscesses are present in a single rat, radioactivity is distributed equally between the two abscesses. However, each of the two abscesses accumulates and retains less radioactivity than does an abscess present in a rat bearing only one such abscess.

In general, one may wonder how it is possible to have antifibrinogen antibody circulating in the presence of plasma fibrinogen. Over a wide range of antibody concentration, I^{131}-antibody preparations of the type used in our studies will rapidly form precipitates with rat fibrinogen if the two are mixed together at approximately equimolar concentrations; however, if the antigen is present in great excess, the antibody-antigen complex is soluble. The longer biological half-life in the blood of the I^{131}-antibody compared with that of fibrinogen has been noted by others (3). Presumably, it is due either to resistance of the complex to normal mechanisms of fibrinogen metabolism, to dissociation of the antibody from fibrinogen as fibrinogen is metabolized, or a combination of these factors.

Summary. An investigation of the effect of orally administered 5% ϵ-aminocaproic acid (EACA) in drinking water on the behavior of intravenously injected I^{131}-fibrinogen and I^{131}-antibody to fibrinogen was carried out in both normal rats and rats with abscesses produced by subcutaneous injection of turpentine. Treatment with EACA had no demonstrable effect on either whole-body retention or blood half-life of either I^{131}-fibrinogen or I^{131}-antibody in normal rats. The most striking effect of EACA was to decrease greatly the rate of removal of I^{131} from the abscess area. Six days after injection of I^{131}-fibrinogen, 11% of the injected I^{131} was still present in single abscesses of EACA treated rats while in non-EACA treated rats the I^{131} retained was less than 1% of the injected dose. Similarly, 6 days after injection of I^{131}-antibody 20% of the injected I^{131} was present in abscesses of EACA treated animals and only 4% in abscesses of rats not receiving EACA. In animals with 2 abscesses, the amount of I^{131} deposited was greater but not double the amount deposited in single abscesses. This evidence supports the hypothesis that EACA is a potent inhibitor of fibrinolysis *in vivo*, as well as *in vitro*.

1. Bale, W. F., Spar, I. L., Goodland, R. L., Izzo, M. J., *Fed. Proc.*, 1961, v20, 58.

2. Mutschler, L. E., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 1019.

3. Day, E. D., Planinsek, J. A., Pressman, D., *J. Nat. Cancer Inst.*, 1959, v23, 799.

Received September 26, 1963. P.S.E.B.M., 1964, v115.

Effect of Thymectomy on Mast Cells in Mice.* (29108)

BRUCE E. WALKER

Department of Anatomy, University of Texas Medical Branch, Galveston

The number of new mast cells produced in adult mice is very small according to radioautographic experiments with thymidine-H^3 (1). Proliferation of existing mast cells(2) could account for the few new mast cells that were identified by their radioactivity(3). There was no evidence of a precursor cell transforming into a mast cell(3). Recently, Ginsberg and Sachs(4,5) reported inducing mast cell formation from thymus cells in tissue culture. They suggested that the thymus may be a normal source of mast cells. The radioautographic experiments on normal mast cell turnover were based on the premise that any proliferating cell population would be labeled by thymidine-H^3 during the course of the experiment. However, since small lymphocytes in the blood can circulate for a long time

* Supported by Houston Endowment, Inc., and Liberty Muscular Dystrophy Research Foundation.