Experiments are now in progress to ascertain the influence of antibiotics and the direct correlation, if any, between intestinal microflora, as measured by fecal and cecal counts, and the ascorbic acid status of the rat on diets containing different levels of protein, sulfadrugs and also lactose.

Summary. 1. The adverse effect of sulfadrugs on urinary excretion of ascorbic acid in the rat and its counteraction by high levels of dietary protein have been substantiated. 2. Lactose, known to enhance proliferation of intestinal microflora, did not in itself raise ascorbic acid levels in the liver or urine when added to high and low protein diets at 10% level. However, it abolished the adverse effects of the sulfadrugs at 2% level which again was reversed by increasing the sulfadrug level to 4%. 3. Supplementation of the diet with all the known vitamins failed to reverse the effect of the sulfadrug.

We are thankful to Dr. V. Subrahmanyan, for his interest in the work and to Mr. A. N. Sankaran for statistical examination of the data.

1. Srinivasan, M., Rao, M. V. L., Bhagavan, H. N., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 615.

2. Roe, J. H., Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.

3. Chatterjee, I. B., Kar, N. C., Ghosh, N. C., Guha, B. C., *Arch. Biochem. Biophys.*, 1960, v86, 154.

4. Caputto, R., McCay, P. B., Carpenter, M. P., *Am. J. Clin. Nutr.*, 1961, v9, No. 4, Part II, p61.

5. Schwartz, M. A., Williams, J. N., Jr., *J. Biol. Chem.*, 1952, v194, 711; v197, 481; v198, 271.

6. Schwartz, M. A., de Salegui, Miriam, Williams, J. N., Jr., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 858.

7. Evans, C., Conney, A. H., Trousof, N., Burns, J. J., *Biochim. et Biophys. Acta*, 1960, v41, 9.

8. Asenjo, C. F., Quintana, M. L., Lebron, A. P., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 561.

Received September 13, 1962. P.S.E.B.M., 1963, v112.

Effects of Epsilon-Amino-Caproic Acid on Prolactin-Inactivating and Fibrinolytic Activities of Streptokinase-Activated Plasminogen.* (28181)

THOMAS F. HOPKINS† AND JOSEPH MEITES‡
Department of Physiology and Pharmacology, Michigan State University, East Lansing

Previous reports have indicated that several protein hormones could be inactivated by blood plasma(1-4) or serum(5) from various mammalian species, and that the degree of inactivation, in some instances, could be increased by streptokinase (SK), a product of bacterial origin(3). SK has been found to be a highly effective activator of the fibrinolysin (plasmin) system of blood, but has not been found to activate other enzyme systems. Thus, some degree of specificity exists between SK and fibrinolysin. Fibrinolysin(5,6) and SK-activated plasminogen(3) have likewise been reported to inactivate protein hormones. The purpose of the studies reported here was to determine the relationship of fibrinolytic activity(FA) to prolactin-inactivating activity (PIA) of SK-activated plasminogen, and to determine whether either activity could be altered by (a) e-aminocaproic acid (EACA), a specific inhibitor of fibrinolytic activity of SK-activated plasminogen, or by (b) bovine antifibrinolysin (AFL), without influencing the other activity.

Methods. Purified ovine prolactin§ with an activity of 15 IU per mg was used. The prolactin was dissolved in distilled water to a concentration of 5.0 mg prolactin per ml solution. One-tenth ml (0.5 mg) prolactin solution was added to each flask which contained the incubation medium in Krebs-Ringer phos-

* Published with approval of Mich. Agr. Exp. Station as Journal Article No. 3067.

† Present address: Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

‡ Supported in part by USPHS grant.

§ Supplied by Endocrinology Study Section, N.I.H.

TABLE I. Effects of Antifibrinolysin and E-Aminocaproic Acid on Prolactin-Inactivating And Fibrinolytic Activities of Streptokinase-Activated Plasminogen. (0.5 mg prolactin used in each experiment.)

Treatment	No. pigeons used/assay	Avg rating of pigeon crops: Untreated prolactin	Avg rating of pigeon crops: Exp prolactin	Loss of prolactin activity: mg	Loss of prolactin activity: %	Lysis of fibrinogen heated fibrin plate, mm² (10.0 λ incubation medium)
5.0 mg plasminogen	10	1.52	1.08	.15	29	36
5.0 mg plasminogen 2000 U SK	10	1.75	.87	.25	50	75
5.0 mg plasminogen 2000 U SK 100 U AFL	5	1.75	1.66	.03	5	0
5.0 mg plasminogen 5.0 mg E-Aminocaproic acid 2000 U SK	5	1.60	.83	.24	48	158
5.0 mg plasminogen 50.0 mg E-Aminocaproic acid 2000 U SK	5	1.50	.75	.25	50	132

SK = streptokinase AFL = antifibrinolysin

phate buffer of pH 7.2. Human plasminogen‖ (23.4 caseinolytic units (CU) per mg N) was not readily soluble at neutral pH; therefore, it was incubated as a fine suspension. Where indicated, plasminogen (5.0 mg) was incubated 10 minutes with EACA in 0.7 ml Krebs-Ringer phosphate buffer, after which 0.2 ml (2000 U) SK and 0.1 ml (0.5 mg) prolactin were added. Total incubation volume was 1.0 ml. The mixture was incubated for 2 hours at 37.5°C in a Dubnoff metabolic shaking incubator. As a control, standard prolactin was added to Krebs-Ringer phosphate buffer and incubated simultaneously with the plasminogen. After incubation an aliquot of each sample was diluted 10-fold with 0.9% saline solution to give an initial prolactin concentration of 0.05 mg/ml. Antifibrinolysin¶ was dissolved in Krebs-Ringer phosphate buffer to a concentration of 500 units per ml solution. A volume of 0.2 ml (100 units) was used to inhibit fibrinolysin activity.

Prolactin activity was assayed in mature White Carneau pigeons by the method of Reece and Turner(7). Specific details have been reported(5). FA of the undiluted incubation preparations was determined by the heated plate method of Lassen(8). The method of preparing the fibrin plate(9) was modified so that 0.2% solution of human fibrinogen was used(9). One drop (10 λ) of each preparation was put on the fibrin plate and the plate was incubated for 18 hours at 34°C. The area of the digested fibrin served as an index of enzyme activity.

Results. Data presented in Table I show that incubation of prolactin with 5.0 mg plasminogen resulted in 29% loss of prolactin activity. In the presence of 2000 U SK the loss of prolactin was increased to 50%. Lysis of fibrin caused by 10 λ of these preparations was 36 mm² for plasminogen and 75 mm² for SK-activated plasminogen.

FA and PIA were completely inhibited by 100 U bovine antifibrinolysin. EACA (AR grade) at 5 and 50 mg levels increased FA, but did not similarly increase PIA.

Discussion. The data presented show that FA and PIA of SK-activated plasminogen do not appear to be directly related. An increase in FA was not accompanied by a corresponding increase in PIA when EACA was used. It is not possible to determine whether the two activities were due to FA alone or if they represent more than one active site on the enzyme molecule. It is possible that other enzymes present in trace amounts could have been influenced by streptokinase as well as by EACA. Sakar(10) reported that fibrinolytic activity of plasmin could be inhibited by

‖ We wish to thank Dr. J. Sgouris of Mich. State Health Labs. for plasminogen.

¶ A Parke-Davis product kindly supplied by Dr. Walter Seegers, Wayne State Univ., Detroit, Mich.

EACA without interfering with fibrinogenolytic activity; ovomucoid inhibited fibrinogenolytic activity but did not appreciably inhibit FA. Hopkins, Terner and Pincus(11), by use of an activator prepared from human blood, converted plasminogen to an enzyme possessing ACTH-inactivating activity but without FA. Thus, it seems reasonable to assume that the enzyme activity of activated plasminogen might be determined by the inhibitors and/or activator employed.

EACA above .06 M concentration has been reported competitively to inhibit FA of SK-activated plasminogen, whereas, below .06 M it enhances fibrinolytic activity(12). In our experiments, the failure of EACA effectively to inhibit FA at 0.26 M (50.0 mg, Table I) could be due to the excessive amount of activator employed in the system (2000 U SK).

Summary. Ovine prolactin (0.5 mg) was incubated for 2 hours at 37.5°C with human plasminogen with and without e-aminocaproic acid (EACA). Fibrinolytic activity (FA) and prolactin-inactivating activity (PIA) were increased by streptokinase (SK). EACA at 0.026 M (5.0 mg) and 0.26 M (50.0 mg) increased FA but did not increase PIA. Antifibrinolysin (100 U) completely inhibited both activities.

1. Reiss, M., Badrick, R. E., Halkerston, I. D. K., Plaice, C., *Nature,* 1951, v168, 206.

2. Pincus, G., Hopkins, T. F., Hechter, O., *Arch. Biochem. and Biophys.,* 1952, v37, 408.

3. Mirsky, I. A., Perisutti, G., Davis, N. D., *J. Clin. Invest.,* 1959, v38, 14.

4. Meakin, J. W., Nelson, D. H., *Endocrinology,* 1960, v66, 73.

5. Hopkins, T. F., Meites, J., Ratner, A., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 140.

6. White, W. F., Gross, A. M., *J. Am. Chem. Soc.,* 1957, v79, 1141.

7. Reece, R. P., Turner, C. W., *Mo. Agr. Exp. Res. Bull.* No. 266, 1937.

8. Lassen, M., *Acta Physiol. Scand.,* 1952, v27, 371.

9. Astrup, T., Mullertz, S., *Arch. Biochem. Biophys.,* 1952, v40, 346.

10. Sakar, N. K., *Activators and Inhibitors of Fibrinolysis,* in Fourth Internat. Cong. Biochem., Pergamon Press, 1961, v10, p165-171.

11. Hopkins, T. F., Terner, C., Pincus, G., *Endocrinology,* 1961, v69, 728.

12. Alkjaersig, N., Fletcher, A. P., Skerry, S., *J. Biol. Chem.,* 1959, v234, 832.

Received October 15, 1962. P.S.E.B.M., 1963, v112.

Relation Between Erythropoietin Plasma Level and Oxygen Requirements.* (28182)

JEAN-PIERRE NAETS† (Introduced by J. H. Lawrence)

Donner Laboratory of Medical Physics, Lawrence Radiation Laboratory, University of California, Berkeley

It has been postulated that red cell production is regulated by the plasma level of erythropoietin and that production of this hormone is regulated by the balance between tissue oxygen supply and demand(1,2). The titer of erythropoietin in plasma increases when oxygen supply is reduced. It has been shown that in starved(3) and in hypophysectomized(4,5) animals the oxygen requirement is reduced. Very little information is available concerning the erythropoietin production in conditions where a reduced oxygen supply is associated with a reduced oxygen need.

This is a study of the effects of hypoxia, cobalt, or anemia on the titer of erythropoietin in plasma of normal, hypertransfused, starved, or hypophysectomized rats.

Material and methods. Groups of 5 to 12 hypophysectomized, starved, or polycythemic male rats of the Sprague-Dawley strain (weighing between 170 and 220 g) were subjected to 3 known stimuli of erythropoietin production: hypoxia, cobalt, or anemia. In each experiment a group of normal animals

* Supported in part by U. S. Atomic Energy Commission.

† Present address: Brugmann Hospital, Brussels, Belgium.