cytoplasm. The doughnut-shaped nucleus of the normal eosinophile breaks down into fragments that no longer stain with Giemsa and the cytoplasmic granules become densely packed to form masses that stain intensely with eosin or phloxine. Shrinkage of the cell may accompany these reactions. These changes are seen especially well in peritoneal fluid and have been described in more detail elsewhere(1). Following the injection of epinephrine, there is an immediate increase in the numbers of these degenerating elements and fragments in peripheral blood (after 45 min: 11.2 $\pm$ 0.70; P $<$0.01), and this rise is maintained throughout the duration of the peripheral eosinopenic state (at 4½ hr: 22 $\pm$ 9.2; P $<$0.05). The data are given in Fig. 1, where the percentage changes of normal and degenerate eosinophiles in peripheral blood are graphed with respect to time after injection.

Careful study of the counting chamber samples suggests strongly that the small eosinophiles arise from the fragmentation of the degenerating eosinophilic leukocyte.

This work lends further support to our contention that eosinophilic cell destruction is a generalized reaction occurring throughout most of the body and that it constitutes an integral part of the mechanism leading to the eosinopenia caused by adrenal hormones and by stress. The present experiments correlate well with our studies on peritoneal and pleural fluids(1,2). Preliminary experiments with cortisone acetate have yielded results similar to those reported here with epinephrine.

Summary. Small numbers of degenerating eosinophilic leukocyte fragments are encountered normally in peripheral blood as well as in other body fluids of the rat. Following epinephrine or cortisone, there is a marked increase in the numbers of eosinophilic leukocyte fragments in peripheral blood, and this increase is maintained throughout the duration of the developing eosinopenia.

1. Padawer, J., and Gordon, A. S., *Endocrinology,* in press.

2. ———, Program of the 34th Meeting of the Endocrine Society, June 1952, p27.

3. Muehrke, R. C., Lewis, J. L., and Kark, R. M., personal communication.

4. Godlowski, Z. Z., *J. Endocrinology,* 1952, v8, 102.

5. Randolph, T. G., *J. Lab. and Clin. Med.,* 1949, v34, 1696.

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Effect of Tyrothricin Upon Fibrinolysis Mediated by Streptokinase. (19698)

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The proteolytic dissolution of a fibrin clot, mediated by streptokinase, has been shown by Neter(1) to be inhibited by tyrothricin. These observations were made using whole human plasma and whole cultures of streptococci or crude filtrates. The lytic action, however, has since been shown to be the resultant of 2 simultaneously occurring reactions: 1) Streptokinase + Plasminogen → plasmin; 2) Plasmin + Fibrin → lysis(2). In view of the present clinical use of the fibrinolytic activity of streptokinase it appeared to be of interest to determine, using a more defined system, which of these reactions is inhibited by tyrothricin.

Materials and methods. 1) *Streptokinase.* Partially purified streptokinase preparations derived from culture filtrates of a fibrinolytic strain of *Streptococcus hemolyticus** were used in these studies. 2) *Plasminogen.* Fraction III, obtained from human plasma by the Harvard method of fractionation(3), was prepared by Dr. W. Baumgarten, of these laboratories. 3) Armour's Bovine *Fibrinogen* and Parke Davis Bovine *Thrombin* were used to form the standard fibrin clot. 4) *Streptokinase assay* was performed by the method of

* *Streptococcus hemolyticus* H46A was obtained through the courtesy of Dr. R. L. Christensen of the New York University Medical School.

TABLE I. Inhibition of Streptokinase Activity by Tyrothricin.

Streptokinase preparation	Tyrothricin addition, μg	Time, min	Streptokinase assay
55A	50	0	320 units
	50	30	60
	50	60	27
	Alcohol	60	280

TABLE II. Effect of Tyrothricin-Treated Streptokinase on Plasmin Formation.

Exp.	Units plasmin: Control streptokinase*	Units plasmin: Tyrothricin-treated streptokinase†
1	100	56
2	136	54
3	112	75

* 100 units streptokinase were incubated with 400 units plasminogen.

† 100 units streptokinase incubated with 100 μg tyrothricin before addition of 400 units plasminogen.

Christensen(4), and *plasmin* was determined by a modification of Christensen's plasminogen assay(4). One unit of plasmin is that amount in 0.5 ml of sample that will cause the lysis of a standard fibrin clot in 30 minutes at 35°C. 5) *Tyrothricin.* Wallerstein's tyrothricin was dissolved in alcohol.

Results. Effect of tyrothricin on streptokinase activity. When tyrothricin was incubated with streptokinase for 30 minutes at 35°C, the amount of recoverable streptokinase dropped sharply (Table I). The test was repeated with several preparations of streptokinase, and the degree of inhibition observed varied from 60 to 90%. Although a complete study of the time of inactivation was not made, it was found that almost maximal inactivation occurred within the first 30 minutes of incubation.

Since the tyrothricin was placed into solution in alcohol, the possible inhibitory effect of the added alcohol on this system was determined by incubating the streptokinase with alcohol alone. The data show that, although there was a slight decrease in streptokinase unitage with alcohol alone, the inhibition caused in the same time period by the alcoholic tyrothricin solution was 90% greater.

Tyrothricin-treated streptokinase and plasmin formation. Streptokinase incubated with plasminogen will induce maximal plasmin formation within a 5-minute period(5). However, 100 units streptokinase pre-incubated with 100 μg tyrothricin for 30 minutes were able to activate only 40-70% of the plasmin activated by the normal control streptokinase (Table II).

Tyrothricin-treated plasminogen and plasmin formation. When plasminogen was incubated with tyrothricin for 30 minutes before activation with streptokinase there was no inhibition of plasmin formation (Table III).

Tyrothricin and plasmin activity. Plasmin was formed by the incubation of streptokinase with plasminogen for 5 minutes. The addition of tyrothricin to an aliquot of the preformed plasmin 30 minutes before the determination of the plasmin activity did not induce a significant loss in plasmin activity (Table IV).

Discussion. The pre-incubation of streptokinase with tyrothricin before reaction with plasminogen induced a decrease in streptokinase activity. This was seen in both the streptokinase recovery assays and in the formation of plasmin with the treated streptokinase. However, when tyrothricin was mixed with plasminogen first, there was no effect on the streptokinase activation of plasmin. This suggests that the plasminogen and tyrothricin combine in such a manner as to prevent the streptokinase inhibition by the antibiotic. When preformed plasmin was placed in contact with tyrothricin normal lytic activity oc-

TABLE III. Plasmin Formation from Tyrothricin-Treated Plasminogen.

Tyrothricin	Units plasmin: Exp. 1	Units plasmin: Exp. 2
None*	126	144
100 μg†	128	156

* 400 units plasminogen activated by 100 units streptokinase.

† 400 units plasminogen incubated for 30 minutes with tyrothricin before activation with streptokinase.

TABLE IV. Effect of Tyrothricin on Plasmin Activity.

Tyrothricin	Units plasmin: Exp. 1	Units plasmin: Exp. 2
None	64	104
50 μg	52	96

curred: streptokinase had already completed the activation of plasminogen and was no longer available for inhibition by tyrothricin. Since there is no inhibition of the proteolytic activity of the plasmin, tyrothricin must act on streptokinase, preventing the activation of the plasminogen.

A study also has been made of the effect of several antibiotics and chemotherapeutic agents on the activation of plasminogen by streptokinase, and on the lysis of the fibrin clot by plasmin. The compounds examined included penicillin, dihydrostreptomycin, polymyxin B, bacitracin, and sulfamerazine. All were tested at several concentrations of the active agent. No inhibition of either reaction was apparent with any of these compounds.

Summary. 1. Tyrothricin, or one or more of its components, reacts with streptokinase to prevent the activation of plasminogen and formation of plasmin. 2. Treatment of plasminogen with tyrothricin does not inhibit the activation of plasminogen by streptokinase. Plasminogen probably reacts with tyrothricin, preventing the inhibition of the added streptokinase by the antibiotic. 3. Tyrothricin does not inhibit the fibrinolytic activity of plasmin. 4. Penicillin, dihydrostreptomycin, polymyxin B, bacitracin, and sulfamerazine did not inhibit the activation of plasminogen by streptokinase nor the fibrinolytic activity of the plasmin.

1. Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 163.

2. Christensen, L. R., *J. Gen. Physiol.*, 1945, v28, 363.

3. Oncley, J. L., *et al.*, *J. Am. Chem. Soc.*, 1949, v71, 541.

4. Christensen, L. R., *J. Clin. Invest.*, 1949, v28, 163.

5. Wasserman, A. E., Manuscript in preparation.

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Toxicity of Mono Methyl Ether of Dipropylene Glycol (Dowanol 50B) in Studies on Auricular Fibrillation. (19699)

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A reliable method for the production of auricular fibrillation in intact animals would be useful in certain investigations. A recent report(1) on the consistent ability of the mono methyl ether of dipropylene glycol (Dowanol 50B) to provoke this arrhythmia in acute experiments on the anesthetized dog led us to inquire whether this compound could be used as a fibrillating agent in experiments repeated in a given dog on different days. The oral lethal dose was reported to be about 7.5 ml/kg (intravenous lethal dose not given) and the fibrillating dose 0.6 ml/kg intravenously or higher. Since recovery from sublethal dose administration was said to be complete, it seemed probable that Dowanol 50B could be used repeatedly as desired. We injected Dowanol 50B intravenously into 4 normal dogs in an attempt to determine whether the minimal dose producing auricular fibrillation in a given animal remains constant on different days. Such constancy of minimal dose has been reported to exist for repeated trials in acute experiments on a single day(1).

Methods. Five experiments were performed in 4 dogs. The animals each received 30 mg/kg pentobarbital sodium intravenously, and a tracheal catheter was inserted. Injections of Dowanol 50B were given rapidly into a foreleg vein while continuous recording of lead 2 was made with a direct writing electrocardiograph. Intervals of 10 to 20 minutes were allowed between injections. Because Dowanol 50B depresses respiration, dogs 1 and 2 were maintained on gentle positive blast respiration throughout, while dogs 3 and 4 were so maintained only after each injection, and were allowed to return to spontaneous respiration before a next injection was given.

Results. We found Dowanol 50B too lethal