Eosinopenia and Degenerating Eosinophilic Leukocytes in Blood.* (19697)

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A mechanism has been described(1) for the eosinopenia that follows the administration of adrenal hormones or the application of stress. The possibility that eosinophiles undergo destruction in the fluid compartments of the body is supported by the finding that different forms of degenerating eosinophilic leukocytes can be observed in the peripheral blood and peritoneal and pleural fluids of the rat under normal and experimental conditions(1,2). Similar observations have been made with human blood *in vitro*(3,4); likewise the destructive influence of adrenal hormones upon eosinophiles has been noted within ligated blood vessels of the dog(4). The time course of the changes in numbers of eosinophiles in the peripheral blood of the rat treated with epinephrine is presented in more detail in this report.

Materials and methods. Six normal female rats (175-200 g) of a modified Long-Evans strain were injected subcutaneously with 1 ml of a freshly prepared 1:5000 solution of epinephrine (Parke-Davis adrenalin chloride) in 1% saline. Tail blood was obtained under light ether anesthesia immediately before and at various times following the injection and diluted with the phloxine-methylene blue glycol stain described by Randolph(5). Since relatively few eosinophiles are detected normally in blood and fewer yet during the eosinopenic state, it was found necessary to count the cells in a larger volume of blood. Standard white cell pipettes were used; blood was drawn to the 1.0 mark and, after dilution, was delivered into both sides of 2 Levy counting chambers and the cells within the entire ruled areas were counted. The lowest number of eosinophiles actually observed at the height of eosinopenia was 32, with as many as 400 cells being present in the early stages of the experiment. As reported previously(1), the degenerating eosinophilic leukocytes are smaller than the normal eosinophiles and are visualized easily in the counting chamber (120 × magnification). Both normal and degenerate eosinophiles were enumerated in each blood sample.

Results and conclusions. Degenerating eosinophilic elements, which we refer to as "small eosinophiles," are observed normally in rat peripheral blood, but in very small numbers (mean ± standard error: 2.2 ± 0.76). The small eosinophilic forms arise from normal eosinophilic leukocytes through degenerative processes involving both the nucleus and

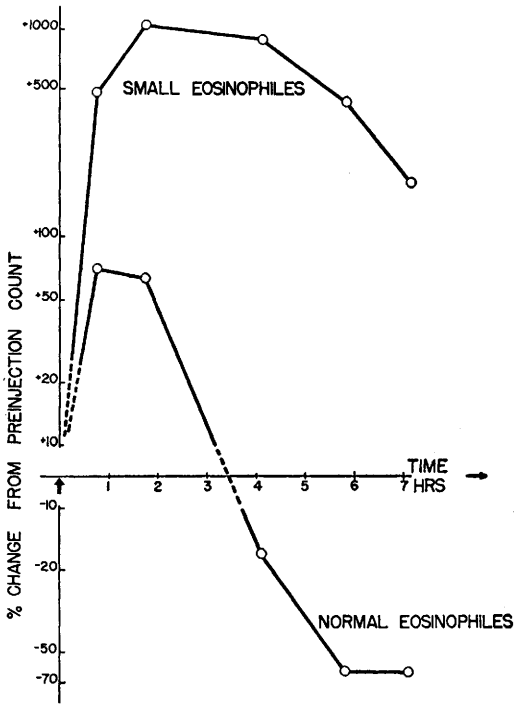

FIG. 1. Variation in the numbers of normal and of degenerating eosinophiles following epinephrine injection. The ordinate is plotted on a logarithmic scale. Note that the numbers of small eosinophiles remain elevated even during the phase of eosinopenia.

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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.

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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

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