

TABLE III. Summary of Analysis of Variance.

Source of variation	D.F.	Var- iance	F	F. _{.05}	F. _{.01}
Between mice	43	99122.62	3.13	<1.50	<1.76
Between exp.	4	21104.88	7.24	<2.45	<3.48
Pooled residual	172	2914.11			

variable. An experimental design providing for all possible protection of experimental animals against extraordinary stimulation has great merits. Animals with no past history of venisection, *e.g.*, can be used whenever comparable populations of experimental animals are available. Statistically significant groups of animals can then be studied at several time points if need be. However, the other possible investigative design, involving the use of animals exposed to the same number of venisections, is often a necessary concession to practicability. It is recognized, however, that this second procedure is open to some criticism. It would approximate control of the stimulation only if mice subjected to venisection could be handled identically. And even if this were feasible it would not necessarily follow that two groups of experimental mice will react identically to venisection and/or the handling associated with this operation.

If eosinophil counts are to be dealt with as a quantitative variable, control of the past history of the animals employed will have to be established, in addition to scrupulous care

for the maintenance of standardized environmental(3) and temporal(2) circumstances. It is conceivable that effects on this variable could then be evaluated as effects on a fundamental physiological cycle in mice(4) and in man(5,6).

Summary. The numbers of circulating eosinophils in the tail blood of mice have been determined at intervals of several days. A decreasing trend in mean number of eosinophils is noted with repeated venisections for various stocks of mice. The procedural implications of the finding are discussed in the light of related data. Control of the past history of experimental animals appears to be necessary if eosinophil counts are to be evaluated quantitatively.

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1. Halberg, F., Bittner, J. J., and Visscher, M. B., *Blood, J. Hematology*, 1951, v6, 832.
2. Halberg, F., and Visscher, M. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v75, 846.
3. ———, *Am. J. Physiol.*, in press.
4. ———, *Endocrinology*, in press.
5. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., and Block, F., *J. Lancet*, 1951, v71, 312.
6. Halberg, F., *J. Lancet*, 1953, v73, 20.

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Degenerative Forms of Eosinophilic Leukocytes in Lymphoid Organs.* (20054)

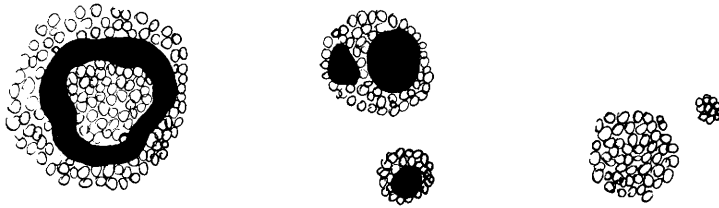
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Recent reports have dealt with the fate of the eosinophilic leukocyte following various stimuli which reduce their numbers in the peripheral blood stream. Padawer and Gordon (1,2) have described the process of breakdown

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of eosinophils in peripheral blood and in the pleural and peritoneal fluids of rats treated with epinephrine or cortisone. It has also been reported(3) that significant decreases occur in the numbers of eosinophils following their incubation *in vitro* with cortisone. The present report provides evidence that eosinophils are found normally in varying stages of breakdown in lymph nodes and that lowered



Figures are diagrammatic representations of normal and degenerative forms of eosinophils.
 FIG. 1. Normal eosinophil. FIG. 2. Eosinorrhexocytes. FIG. 3. Eosinomeres.

barometric pressures will increase the relative numbers of degenerated eosinophils in these organs.

Materials and methods. Adult male rats of a modified Long-Evans strain and weighing 265-400 g were employed. Eight rats were exposed to a simulated altitude of 25,000 ft. (282 mm Hg) for 6 hours in a specially constructed low pressure chamber. During this time they received food and water *ad libitum*. At the end of this period of exposure, they were anesthetized and decapitated. Eight untreated rats served as controls. Touch prints were made of the ileo-cecal lymph nodes. These organs were employed because they were found to contain greater numbers of eosinophils than did lymph nodes in other locations. Samples of these nodes were also fixed in Zenker-formol. Similar procedures were repeated for the spleen, thymus and other lymphoid tissues. The imprints were stained with Giemsa and the sections with hematoxylin and chromotrope 2-R(4). Peripheral eosinophil counts were made by the Randolph (5) chamber method. The evaluation of eosinophil destruction was standardized by examining the touch prints of the ileo-cecal lymph node closest to the cecum. At least 200 eosinophilic elements in this node were counted for each animal and classified as follows(6): A) Eosinophils. This includes all nucleated eosinophils possessing either a "ring form" nucleus (Fig. 1) or a solid nucleus with a chromatin network that appears normal. B) Eosinorrhexocytes. These bodies are smaller than those in the first category. The nuclear material is reduced in size and usually presents a laked appearance when stained with Giemsa. One or more nuclear fragments may be present (Fig. 2). C) Eosinomeres. These eosinophilic bodies are anuclear. They are

usually spheroid and are composed of granules that are indistinguishable from those seen in the eosinophils of categories A and B. Both the size and extent of granulation of the eosinomeres are variable (Fig. 3).

Results and conclusions. Male rats weighing more than 200 g were employed in these experiments because of the greater numbers of eosinophils detected in their ileo-cecal lymph nodes than in those of smaller animals (100-180 g). Considerable numbers of eosinomeres were apparent in untreated rats and in rats exposed to the reduced pressures. They were seen in touch prints, sections, smears and in suspensions observed in a warm chamber. The size of eosinomeres varied from approximately $1\ \mu$ to $10\ \mu$ in diameter.

Examination of $5\ \mu$ sections of ileo-cecal lymph nodes often revealed the presence of eosinophils in the capsular and adjacent cortical areas. These eosinophils frequently appeared in clumps and many of them displayed marked degeneration. In the connective tissue capsule the eosinophils were spindle-shaped and arranged parallel to the capsule.

A peripheral eosinopenia was observed in the rats subjected to the lowered barometric pressures for 6 hours. In addition, a marked "shift to the right" was noted in the percentages of the 3 categories enumerated for the ileo-cecal lymph nodes (Table I). Touch prints and supravital studies have suggested that these eosinophils undergo destruction through budding and degranulation. Despite signs in touch prints of phagocytosis of lymphoid and erythroid cells by macrophages in the lymph nodes, only rarely was the ingestion of eosinophilic elements noted. Evidence of eosinophil destruction similar to that in the ileo-cecal lymph nodes has been observed also in the spleens, thymuses, and thymic, renal

TABLE I. Mean Percentages ($\pm$ Stand. Errors) of Eosinophilic Forms in Ileo-cecal Lymph Nodes of Rats.

	Eosinophils	Eosinorrhexicytes	Eosinomeres
Controls (no pressure)	65.1 $\pm$ 4.8	.4 $\pm$.2	34 $\pm$ 6.5
Experimentals (280 mm Hg 6 hr)	32.6 $\pm$ 3.2	15.3 $\pm$ 3.6	52.3 $\pm$ 4.4
P values*	<.01	<.01	<.02

* Probability values obtained from the distribution of Fisher's t. The 3 values are considered significant.

and lumbar lymph nodes. Examination of the colon, liver and lungs has revealed eosinophils of only the type A variety. Immature eosinophils have been seen occasionally in smears of spleen suspensions; however, these number fewer than 0.1% of the total eosinophils seen.

The question remains open as to whether an active process of eosinophilic leukocyte destruction occurs within the lymphoid organs or whether the degenerated eosinophilic forms represent bodies filtered out by the nodes. In addition, the mechanism for the breakdown of the eosinophil as seen in the rat exposed to lowered barometric pressures is still to be elucidated. Experiments are under way to determine the precise role played by the adrenal cortex in this phenomenon.

Summary. Degenerative forms of eosinophilic leukocytes are found commonly in ileo-cecal lymph nodes and in other lymphoid tissues of the rat. The relative numbers of these degenerative forms are increased in rats subjected to lowered barometric pressures.

1. Padawer, J., and Gordon, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1952, v80, 581.
2. ———, *Endocrinology*, 1952, v51, 52.
3. Muehrcke, R. C., Lewis, J. L., and Kark, R. M., *Science*, 1952, v115, 377.
4. Lendrum, A. C., *J. Path. and Bact.*, 1944, v56, 441.
5. Randolph, T. G., *J. Lab. and Clin. Med.*, 1949, v34, 1696.
6. Padawer, J., and Gordon, A. S., in press.

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Influence of Small Doses of Heparin on Fat Tolerance Curves *in vivo* and *in vitro*.* (20055)

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Hahn's(1) discovery that lipemic serum could be cleared by heparin has stimulated work on the nature of the mechanisms involved. Recently, Anderson and Fawcett(2) reported that heparin added to lipemic serum *in vitro* did not clear the serum, but when a post-heparin serum sample was mixed with the lipemic serum, clearing of the lipemic serum occurred. The material present in the post-

heparin serum, was called "antichylomicrone-mic substance." The nature of this clearing factor has been somewhat clarified by Anfinsen and co-workers(3) who isolated a serum protein (Cohn IV-I) which in the presence of heparin and a tissue factor, resulted in the formation of the "clearing factor" (Cohn III 1, 2, 3), and they were further able to demonstrate also that once formed, the complex was activated by a protein in another serum protein fraction (Cohn III-0). Block(4) and co-workers have reported that great differences exist in the degree to which sera of different

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