Persistence of Eosinophil Rhythm after Splenectomy in Rats.* (21114)

F. HALBERG, P. ALBRECHT, AND H. A. ZANDER.

From the Department of Physiology and Division of Periodontology, University of Minnesota, and the Cambridge State School and Hospital, Cambridge, Minn.

The 24-hour periodicity in number of circulating blood eosinophils seems to be a feature of the physiology of several mammals and the literature on this subject has been discussed(1). It is the purpose of this report to suggest that the eosinophil rhythm may persist in the absence of the spleen.

Materials and methods. Male, Line 3 Black rats, maintained in the Department of Biochemistry at the St. Paul Campus of the University of Minnesota were used. From weaning and throughout the study Purina Fox Chow and tap water were available to the rats *ad libitum*. For 3 weeks prior to as well as during the study the rats were kept singly housed in a room maintained at 25.5 ± 0.5°C; this room was kept illuminated from 06 to 18 and dark from 18 to 06. The rats were 5 months of age at the time of operation and showed no sign of disease. Splenectomy was carried out on 12 rats, ether anesthesia being used. Another group of 12 rats was subjected to a sham-operation. Two independent subgroups, composed of 6 rats each, from the splenectomized group as well as from the sham-operated group were used for the sampling of blood at the 2 times of day chosen for investigation; namely, between 09 and 11 (day) and between 21 and 23 (night). The procedures employed for eosinophil counts have been described(2).

Results. It may be seen from Fig. 1, first, that on the average the day counts were consistently higher than the corresponding night counts, for groups of unoperated or sham-operated rats as well as for splenectomized rats. It is further apparent from Fig. 1 that the mean eosinophil counts of both operated groups show an increasing trend up to 77 days after operation; the mean counts are again lower at 112 days after operation.

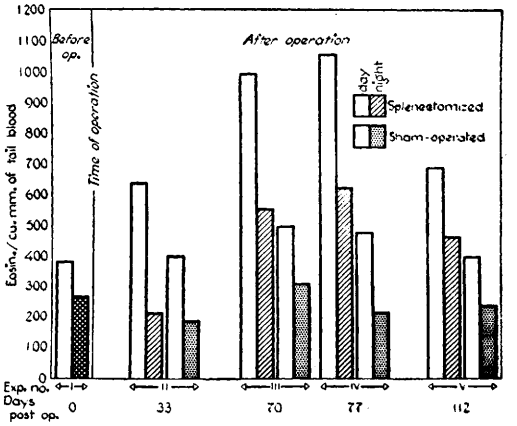

FIG. 1. Eosinophil levels at 2 times of day on Line 3 Black rats, some splenectomized, others subjected to a sham-operation. Note the persistence of eosinophil rhythm after operations.

It may be finally seen from Fig. 1 that the extent of postoperative eosinophilia is larger for rats subjected to splenectomy, in comparison to rats subjected to a sham-operation.

Discussion. The observation of eosinophilia following spleen removal is in keeping with corresponding earlier observations on several species, including sporadic observations on man(3), except for the earlier development and earlier regression of the phenomenon in the rat. Species differences in the times of development and of regression of eosinophilia after splenectomy may be related to differences in life span. The times of development and of regression of changes in eosinophil level, as noted herein, agree well with earlier data on changes in the levels of other circulating leukocytes, after splenectomy on rats(4).

The observation that the day-night differences in mean eosinophil count of rats persist for several months after splenectomy is in keeping with the suggestion that the presence of the spleen is not essential for the maintenance of the eosinophil rhythm, at least as far as the rats studied are concerned.

Summary. Eosinophil counts were made on tail blood obtained at two times of day from

* This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

male Line 3 Black rats kept under standardized circumstances and subjected either to splenectomy or to a sham-operation. Eosinophilia was seen to develop during the first few months after operation and to regress during the fourth month after operation. Irrespective of the degree of eosinophilia, the mean eosinophil counts of the groups of rats studied were higher by day than by night. In the strain of rats studied, the presence of the spleen is not essential for the maintenance of the eosinophil rhythm.

1. Halberg, F., *Lancet,* (U.S.A.), 1953, v73, 20.
2. Halberg, F., Bittner, J. J., and Visscher, M. B., *Blood,* 1951, v6, 832.
3. Ehrlich, P., and Lazarus, A., *Anemia,* in *Normal and Path. Histol. Blood,* Part I, vI, 111, 2nd ed., Rebman Ltd., London, 1910.
4. Palmer, J. G., Kemp, I., Cartwright, C. E., and M. M. Wintrobe, *Blood,* 1951, v6, 3.

Received May 27, 1954. P.S.E.B.M., 1954, v86.

Serologic Characterization of the Fort Bragg Leptospire. (21115)

AARON ALEXANDER, LARUE B. EVANS, HELEN JEFFRIES, CHESTER A. GLEISER, AND ROBERT H. YAGER.

From the Veterinary Division, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D.C.

Outbreaks of a disease that appeared among soldiers stationed on the military reservation of Fort Bragg, N. C. during the summer months of 1942, 1943, and 1944, became known as Fort Bragg or Pretibial Fever(1,2). The infectious agent, isolated by Tatlock in 1944 from the blood of an acutely ill patient and subsequently maintained in various laboratories by serial animal passages, was believed to be a virus until Gochenour *et al.*(3) clearly established the leptospiral etiology of Fort Bragg Fever, and recovered the leptospiral agent from infectious material of the 259th serial hamster passage. Preliminary serologic studies by these workers by means of agglutination-lysis procedures indicated that the Fort Bragg agent was related to the type strain *Leptospira autumnalis,* Akiyami A. This was the first report of a member of the *L. autumnalis* serogroup in the United States. *Classification of leptospires* is based upon stable and specific antigenic characteristics revealed by the agglutination-lysis reaction(4). The genus is divided into "serogroups" of closely related "serotypes." By definition, a serotype includes all strains indistinguishable one from the other on the basis of cross-absorption procedures. Within the serogroup, however, one may find in addition to heterologous serotypes, "complete" and "incomplete" biotypes. These latter may be designated by the antigenic symbols AB and A respectively, indicating that the "incomplete" biotype contains part, but not all of the antigenic components of the "complete" biotype(5). Wolff's(5) proposed classification of leptospires, based upon their cross-agglutination-lysis reactions, groups 36 antigenically distinct leptospiral serotypes into 20 serogroups. This scheme has been tentatively accepted by various leptospiral typing laboratories throughout the world as a working basis in the typing of leptospiral strains. The *autumnalis* serogroup listed in Wolff's scheme is comprised of the following serotypes:

1. *Autumnalis AB* (Type strain Akiyami A) Etiologic agent of autumnal fever in Japan and frequently found in S. E. Asia. 2. *Autumnalis A* (type strain Rachmat). Incomplete "biotype" of *autumnalis AB,* found in S. E. Asia. 3. *Bangkinang* (type strain Bangkinang I). A heterologous member of the *autumnalis* group found in S. E. Asia. With the recent acquisition of the Rachmat and Bankinang strains, an antigenic analysis of the Fort Bragg leptospire was performed using the agglutination-lysis technic.

Methods. Preparation of immune serums. Normal rabbits weighing approximately 3 kg were inoculated intravenously with 1.0 ml, 2.0