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.

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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

TABLE I.
Cross Agglutination-Lysis Reaction of the Fort Bragg and *Autumnalis* Serogroup Strains.

Immune sera	Homologous titer	Antigens			
		Fort Bragg	Akiyami A	Rachmat	Bangkinang I
		×100			
Fort Bragg	1024	1024	1024	256	1024
Akiyami A	1024	1024	1024	64	256
Rachmat	4096	1024	4096	4096	64
Bangkinang I	256	256	256	64	256
andaman	256	—	—	—	—
semarang	256	—	—	—	—
djatzi*	256	—	—	—	—
hyos	256	—	—	—	—
pomona	64	1	1	1	1
djasiman	256	1	—	1	—
sentot	256	16	64	4	16
ballico	64	—	—	1	—
ballum	256	—	—	—	—
canicola	1024	—	—	—	—
ictero. AB	256	1	—	1	—
pyrogenes	256	—	—	—	—
alexi†	1024	—	—	—	—
grippotyphosa	4096	16	—	4	4
borincano‡	256	—	—	—	—
sejroe	1024	—	—	—	—
javanica	256	—	—	—	—

* Member of the serogroup *bataviae*.
† " " " " *pyrogenes*.
‡ " " " " *hebdomadis*.

ml, 4.0 ml, and 6.0 ml of a 5- to 7-day-old live Fletcher's(6) culture of the desired leptospiral strains at 5- to 7-day intervals. Five to 7 days following the last inoculation, the rabbits were exsanguinated and the serums harvested. The serums after Seitz filtration were stored at –20°C. *Antigens employed.* Five- to 7-day-old Schuffner's(7) cultures containing live leptospires were employed as antigens. *Agglutination-lysis test.* Serial 4-fold dilutions were prepared with each serum to be tested using buffered saline (pH 7.4) as diluent and providing serum dilutions of from 1:50 through 1:204,800. To 0.2 ml of each serum dilution in a standard Kahn tube was added an equal quantity of antigen. The tubes were shaken, incubated at 30°C for 3 hours and then examined by (10 X objective and 15 X ocular) dark-ground illumination without the use of cover glasses. Agglutination or lysis, or both, were read as complete, partial, trace, or absent. Titers were expressed as the reciprocals of the highest dilution in which complete agglutination or lysis was observed. *Absorption procedure.* Absorbing antigen was prepared from 7- to 9-day-old leptospiral cultures maintained in Stuart's medium(8) (without glycerol) at 30°C. Cultures were centrifuged at approximately 4000 g in the International Centrifuge PR-1 for one hour in 100 ml cups employing International Centrifuge head No. 845. The supernatant fluid was discarded and the leptospires were resuspended to approximately 1% of the original culture volume with 0.25% formalinized buffered saline (pH 7.4). Four parts of the absorbing antigen were added to one part of a 1:10 dilution of immune serum, placed in a shaker apparatus for two hours, and maintained overnight in a 30°C incubator. In order to avoid further dilution of the serum, subsequent absorptions were performed with centrifuged packed leptospires, obtained by centrifuging the same quantity of antigen suspension used in the first absorption, at approximately 20,000 g for 10 minutes in the International centrifuge PR-1 using 7.0 ml cups and the high speed adapter head, and discarding the supernatant. The absorbing mixtures were centrifuged in the same manner, the supernatant withdrawn and tested by the described agglutination-lysis procedure. Routinely two successive absorptions were performed. Absorptions were considered com-

TABLE II. Cross Agglutinin-Absorptions.

Antiserum Against	Absorbed with	Antigen *aut.* Akiyami A	*aut.* Rachmat	Bangkinang	Fort Bragg
		× 100			
1. *autumnalis* AB Akiyami A	Unabsorbed	256	16	64	256
	1 *aut.* Aki. A†	—	—	—	—
	2 *aut.* Rachmat	64	—	16	64
	3 Bangkinang	256	1	—	64
	4 Fort Bragg†	4	—	—	—
2. *autumnalis* A Rachmat	Unabsorbed	64	64	16	64
	1 *aut.* Aki. A*	—	4	—	—
	2 *aut.* Rachmat	—	—	—	—
	3 Bangkinang	—	4	—	—
	4 Fort Bragg*	—	4	—	—
3. Bangkinang	Unabsorbed	64	16	64	64
	1 *aut.* Aki. A	1	1	16	4
	2 *aut.* Rachmat	64	—	64	64
	3 Bangkinang	—	—	—	—
	4 Fort Bragg*	1	1	16	—‡
4. Fort Bragg	Unabsorbed	64	16	16	256
	1 *aut.* Aki. A†	—	1	1	16
	2 *aut.* Rachmat	16	—	16	256
	3 Bangkinang*	16	1	—	64
	4 Fort Bragg†	—	—	—	—

* Absorbed 3 times.
† " 4 " .
‡ 1st dilution not done, 2nd dilution neg.

plete when no macroscopic agglutination was evident in the absorbing mixture and agglutinins were completely removed by the homologous antigen. If necessary, additional absorptions were performed with packed cells.

Results of the cross agglutination-lysis reactions of the Fort Bragg isolate and members of the serogroup *autumnalis* employed as antigens against a screening battery of type-leptospiral immune rabbit serums, selected on the basis of previous serologic classification studies (9) is shown in Table I. Within the limitations of this procedure, the Fort Bragg strain is indistinguishable from other strains of the *autumnalis* serogroup. However, subsequent antigenic analysis utilizing agglutinin-absorption procedures (Table II) clearly demonstrates that these strains are distinctly heterologous, sharing common major antigens. The agglutinins responsible for the strain specificity are not completely removed when absorbed with any of the other strains, although in several instances there are marked reductions in homologous titers. It is interesting to note that absorption of the Rachmat strain (the "incomplete" biotype) agglutinins with Akiyami A (the "complete" biotype) antigen does not affect a complete reduction of homologous titer.

Discussion. According to Wolff's proposed scheme of leptospiral classification(5) a serogroup is comprised of sterotypes sharing major antigenic components. Wolff "provisionally considered two strains to be heterologous if the antiserum of each strain, after absorption by the other strain, retains at least 10% of its original titer when retested against the homologous strain." This same criterion must necessarily apply to the consideration of "incomplete" and "complete" biotypes.

The leptospiral strains like the members of the genus *Salmonella* show marked serologic heterogeneity. The application of an arbitrary numerical standard may have particular merit in the determination of serotypes especially in view of the fact that the relatively low yield of leptospiral cultures practically precludes any comprehensive antigenic factor analysis. There are, however, certain drawbacks in Wolff's scheme to simplify leptospiral classification, attributable to the many variable factors inherent in agglutination-lysis absorption procedures which affect titers. Quantitative differences in titers may be

markedly affected by the use of formalinized or living cultures, by the age and density of antigens employed in agglutination-lysis procedures(10), by the maximum degree of agglutination and/or lysis accepted as positive and by the particular immune rabbit serum employed(11). It is to be expected that different laboratories employing diversified technics may differ quantitatively in the antigenic evaluation of strains. Until a suitable stable antigen is developed and incorporated into a universally acceptable standardized agglutination-lysis procedure, we have applied Wolff's proposals, with some modifications, to the study of leptospiral serotypes.

The agglutinin-absorption technics employed by Wolff and other European workers differ from those used at the Army Medical Service Graduate School in their initial dilution of all immune serums to a standard titer of 3000, in their employment of a final dilution scheme of 1:10, 1:30, 1:100, 1:300, 1:1000, and 1:3000 and in their use of formalinized test antigens(12). Thus with their dilution scheme, 2 strains are considered homologous if the antiserum of each strain, after absorption by the other strain, is reduced to at least 3⅓% of its original homologous titer, while the retention of at least 10% of the homologous titer is the criterion for heterologous strains. With the technic employed in our studies, we have adopted 1.6% (1/64 of homologous titer) and 6.2% (1/16 of homologous titer) as the breakpoints in the determination of homologous and heterologous serotypes. Our selection of these criteria particularly in view of the serial dilutions employed, is based on numerous absorption studies, and in most of these studies the results have been compatible with the findings of the European workers.

The application of these criteria to the cross agglutinin-absorption results in Table II indicates that the *autumnalis,* Akiyami A strain is an "incomplete" biotype of the Fort Bragg isolate and that contrary to previous reports (5) the Rachmat strain is a heterologous member of the *autumnalis serogroup.* We propose the designation of the Fort Bragg isolate as *L. autumnalis,* Fort Bragg.

Summary. 1. Results of cross agglutination-lysis and cross agglutinin-absorption studies indicate that the etiologic agent of Fort Bragg Fever is closely related to the other members of the leptospiral serogroup *autumnalis.* 2. Agglutinin-absorption studies with all members of the serogroup *autumnalis* reveal that the agglutinins responsible for strain specificity are not completely removed when absorbed with any of the other strains. 3. Classification of these leptospiral strains on the basis of a modified adoption of Wolff's scheme(5) designates *autumnalis* Akiyami A as the "incomplete" biotype of the Fort Bragg isolate and discloses *L. autumnalis A* Rachmat, in addition to *L. bangkinang,* as a heterologous serotype member of the autumnalis group. 4. The Fort Bragg isolate is therefore designated as *L. autumnalis,* Fort Bragg.

1. Daniels, W. B., and Grennen, H. A., *J.A.M.A.,* 1943, v122, 361.

2. Tatlock, H., *J. Clin. Invest.,* 1947, v26, 287.

3. Gochenour, W. S., Jr., Smadel, J. E., Jackson, E. B., Evans, L. B., and Yager, R. H., *Pub. Health Rep.,* 1952, v67, 811.

4. Wolff, J. W., and Broom, J. C., *Riassunta della communicazioni. VI. Congresso internazionella di microbiologie, Rome,* 1953, v2, 424.

5. Wolff, J. W., *Symposium on The Leptospiroses, Med. Sci. Pub.* No. 1, Army Medical Service Graduate School, Walter Reed Army Medical Center, Wash., D.C., 1953, 174.

6. Fletcher, W., *Trans. Roy. Soc. Trop. Med. and Hyg.,* 1927-1928, v21, 265.

7. Gochenour, W. S., Jr., Yager, R. H., Wetmore, P. W., and Hightower, J. A., *Am. J. Pub. Health,* 1953, v43, 405.

8. Stuart, R. D., *J. Path. and Bact.,* 1946, v58, 343.

9. Gochenour, W. S., Jr., and Yager, R. H., *Proc. A.V.M.A.,* 1952, 178.

10. Borg-Petersen, C., and Fagraeus, A., *Acta Path. et microbiol. Scand.,* 1949, v26, 555.

11. Unpublished data from Army Medical Service Graduate School.

12. Schuffner, W., and Bohlander, H., *Zentralbl. f. Bakt.,* 1939, v144, 434.

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