Complement Fixation Tests For Murine Typhus On Small Mammals

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THE IMPORTANCE of commensal rats and their fleas in the epidemiology of endemic typhus was well established in 1931 (*1*, *2*). Following Dyer's report (*3*) on the experimental infection of the woodchuck, meadow mouse, and whitefooted mouse with endemic typhus, Brigham (*4*, *5*) indicated that many species of rodents and other mammals were apparently susceptible to endemic typhus. Sparrow (*6*) recovered a strain of endemic typhus rickettsiae in the house mouse (*Mus musculus*), as did Brigham (*7*) with his experiments on a field mouse (*Peromyscus* sp.).

In view of the wide distribution of endemic typhus in commensal rats in Lavaca County reported by Irons and associates (*8*), inquiry into the possible occurrence of infection in other small mammals seemed worth while. Morlan and co-workers (*9*) used the complement fixation test as an indication of natural infection, but Rickard and Worth (*10*) considered the findings on the wild-caught cotton rat (*Sigmodon hispidus*) to be nonspecific.

Methods

Live animals were captured in 1945 and 1946 near Hallettsville, in southeast Texas. Generally, the ectoparasites were identified while alive. The ectoparasites to be tested for typhus were appropriately pooled and stored on dry ice under glass seal. The animals' brains were removed for tissue infectivity tests and were also stored on dry ice. Tests of the tissues of serologically reactive animals and pools of their fleas aided in evaluating the significance of low titers.

Blood samples were obtained by cardiac puncture soon after capture of the animals. Serums were separated aseptically and were kept at ice-box temperature until examination. Serums were inactivated 30 minutes immediately before testing at 56° C. Each serum was subjected to a quantitative complement fixation test employing endemic typhus rickettsiae. A slight modification of the procedure described by Brigham and Bensgton (*11*) was used. The result was recorded as reactive when a 3+ or greater reaction was obtained with satisfactory controls at a 1:20 or greater dilution of serum.

When a serum was reactive, the corresponding brain suspension was emulsified and inoculated individually into hamsters for evidence of typhus. With negative serologic findings, the brains of animals of the same species, particularly from the same trapping area, were similarly tested in pools of varying number. Pools of fleas from the same host species taken in the same area were similarly tested. Each hamster was bled twice, both before inoculation and 3 or 4 weeks later, and the serums were run in the quantitative complement fixation test. With positive findings, titers tended to be high on the second bleeding. This was the procedure recommended by Plotz, Wertman, and Bennett (*12*) for identifying rickettsial agents isolated in guinea pigs or mice. The utilization of specific complement fixation tests is much more economical than cross immunity and other tests in the guinea pig for identifying endemic typhus rickettsiae. A summary of findings is shown in the table.

The brain tissues of 3 of 17 house mice (*Mus musculus*) taken from 3 of 44 places surveyed yielded typhus rickettsiae. Most of the house mice were trapped on premises in close association with commensal rats. Curiously enough, those trapped in the fields were uniformly negative. Native rats and mice were found strictly in the fields. Tests of the brain tissues from the cotton rat (*Sigmodon hispidus*), the pack rat

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Results of complement fixation tests for typhus fever on animal serums

Mammal species	Total number tested	Percentage reactive	Titer percentage		
			1:20	1:40	Above 1:40
Didelphis virginiana (opossum)	27	6.9	6.9		
Neotoma floridana (pack rat)	14	14.2	7.1	7.1	
Baiomys taylori (field mouse)	101	0			
Sigmodon hispidus (cotton rat)	62	8.0	8.0		
Spilogale indianola (civet cat)	2	0			
Mus musculus (house mouse)	216	7.8	.9	4.6	2.3
Perognathus hispidus (pocket mouse)	3	0			
Peromyscus leucopus (white-footed mouse)	5	0			
Geomys breviceps (gopher)	28	0			
Procyon lotor (raccoon)	13	0			
Reithrodontomys fulvescens (harvest mouse)	9	0			

(*Neotoma floridana*), and the opossum (*Didelphis virginiana*) were uniformly negative.

A pool of 20 fleas (*Ctenocephalides felis*), collected from two opossums at Hallettsville in the summer of 1946 and not previously reported, gave an unequivocal positive test for typhus. These opossums were trapped in an oil mill which had not been dusted with DDT. The opossums showed negative complement fixation tests. Eight pools of fleas collected from negative rats and mice and tested for harborage of typhus rickettsiae gave negative results.

Discussion

Although occasional serums from cotton rats, wood rats, and from an opossum were reactive in the complement fixation test for endemic typhus, the titers were relatively low. However, similar findings on commensal rats probably would have been considered as evidence of infection or immunity to typhus. Low titers and failure to demonstrate the infectivity of brain tissues of cotton rats, pack rats, or opossums cast doubt on the specificity of serologic findings on these animals. The percentage of house mice showing positive serologic findings was also relatively low, but the titers were not invariably low, and endemic typhus rickettsiae were obtained from the brains of three house mice. The relatively few places harboring house mice with evidence of past infection contrasted sharply with findings for the domestic rat (*8*).

The finding of typhus rickettsiae in a pool of fleas collected from the two opossums, and in other pools of fleas collected from nonmurine hosts (*13*, *14*) was perhaps fortuitous, as it is possible that the fleas had acquired the infection from rats. At any rate, these findings do not in any way detract from the primary role of commensal rats in the epidemiology of endemic typhus fever.

Summary

Endemic typhus rickettsiae were recovered from brains of three house mice taken from human habitations and from a pool of 20 fleas collected from two opossums.

House mice trapped in fields were uniformly negative.

Unequivocal evidence of typhus in small mammals other than commensal rats and mice was not obtained.

Serologic findings on the pack rat, cotton rat, and opossum were of doubtful significance.

ACKNOWLEDGMENT

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Public Health Service Staff Announcements

A new post has been created in the Bureau of State Services of the Public Health Service to give over-all direction to field research. **Dr. Justin M. Andrews** will fill the post as an Assistant Surgeon General and Associate Chief of the Bureau. He will direct the programs of applied research in the control programs and at the same time will work with the States in their programs requiring research. The major Public Health Service units involved are the Communicable Disease Center at Atlanta, the Environmental Health Center at Cincinnati, and the Arctic Health Research Center at Anchorage.

Since January 1951, Dr. Andrews has been chief of the Communicable Disease Center, and was deputy chief for 6 years previously. He is a nationally known authority on malaria and other insect borne disease. His experience includes 12 years of university teaching, the direction of Georgia's malaria and hookworm service, and wartime service in Europe and the Pacific. He is a member of the Board of Editors of *Public Health Reports*. Dr. Andrews, a senior scientist, was commissioned in the Public Health Service in 1946.

Dr. Theodore J. Bauer succeeds Dr. Andrews as Chief of the Communicable Disease Center. Dr. Bauer has been chief of the Division of Venereal Disease since May 1948. Before that he was for 5 years the venereal disease control officer of the Chicago Board of Health and medical officer in charge of the Chicago Intensive Treatment Center for venereal disease. Dr. Bauer, a commissioned medical officer, has served in the Public Health Service since December 1934.

Miss Bertha Tiber, Public Health Service commissioned nurse, has been assigned to Tripoli to work with Libyan nurses and other health aides to extend public health nursing services, especially in rural areas. Her assignment is part of the United States technical assistance program. Miss Tiber recently served as chief nurse in the American Zone of Germany and, in 1945, as chief nurse of the Middle East Office at Cairo of the United Nations Relief and Rehabilitation Administration.

Dr. Oswald F. Hedley, 49, chief of the public health office of the Mutual Security Agency, died November 18, 1952. His Public Health Service career began as an intern in 1918 and included research in rheumatic heart disease as well as numerous field assignments here and abroad. In 1947–48 he was medical director of the American Mission for Aid to Greece. Until his association with the Mutual Security Agency in 1950 he served as liaison officer for the Economic Cooperation Administration.