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Recovery of a Rickettsia of the Spotted Fever Group from *Microtus pennsylvanicus* from Virginia. (20950)

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Certain of the small mammals native to North America, particularly rodents and rabbits, have been suspected of serving as reservoirs of Rocky Mountain spotted fever because (a) they are the natural hosts of the tick vectors of this disease; (b) many are experimentally susceptible to infection with spotted fever; and (c) antibodies against spotted fever rickettsiae have been demonstrated in sera from some of these small mammals. However, despite the fact that the disease has been long studied in the United States, no confirmed strains of spotted fever have yet been reported isolated from wild-caught mammals in this country.

During 1952 and 1953 isolation studies were undertaken at the Army Medical Service Graduate School in an effort to detect the presence of spotted fever rickettsiae among small wild mammals collected in known endemic areas in the vicinity of the District of Columbia. The present paper reports the isolation of a strain of rickettsiae belonging to the spotted-fever group from the tissues of a meadow mouse, *Microtus pennsylvanicus*, collected during this study from a suburb of Alexandria, Va.

Materials and methods. One hundred and five small mammals were collected from yards and fields believed to be sources of recent cases of spotted fever in the vicinity of Alexandria, Va., and of Gaithersburg and Laurel, Md. These animals were trapped alive by means of small metal box traps and were returned to the laboratory for rickettsial isolation studies.

Ten percent suspensions of the brain, spleen and liver from each of the wild-caught animals were prepared in sterile saline and inoculated intraperitoneally into male guinea pigs. A portion of the inoculum in each case was streaked on blood agar plates to determine the presence of contaminating bacteria. The test guinea pigs were examined daily for fever (rectal temperature of 104°F or higher) and signs of disease. Those guinea pigs which became febrile 3 or more days following inoculation were routinely sacrificed on the second or third day of fever and transfer of suspensions of their liver, spleen, testes and tunica vaginalis were made intraperitoneally to fresh guinea pigs and mice and into the yolk sacs of 7-day-old embryonated eggs. Impression smears were prepared from the uncut surfaces of spleen and liver and from scrapings of the tunica vaginalis of passage guinea pigs. These preparations were stained by Giemsa's and Macchiavello's methods and examined microscopically for the presence of intracytoplasmic rickettsia-like bodies. When an infectious agent suggestive of rickettsiae was isolated in guinea pigs, convalescent sera drawn from these animals 21 to 30 days after the subsidence of fever were tested for complement-fixing antibodies against spotted fever rickettsiae. Complement-fixation tests were performed according to the methods employed in the Department of Virus and Rickettsial Diseases of the Army Medical Service Graduate School. Sera from passage guinea pigs were titrated in the presence of specific

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washed spotted fever and rickettsialpox antigens which were prepared from the yolk sacs of embryonated eggs infected with the Bitterroot strain of *Rickettsia rickettsii* and the MK strain of *R. akari,* respectively, according to the method of Plotz *et al.*(1). Guinea pigs which had recovered from infection with a suspected rickettsial agent were subjected to challenge inoculations with Bitterroot or Azain strains of *R. rickettsii.* (The Azain strain was isolated from a fatal human case of spotted fever at the AMSGS in 1943, while the Bitterroot strain was obtained from the Rocky Mountain Laboratory in Montana).

Results. Rickettsial isolation attempts were made from the following animals during the course of this study: 65 meadow mice (*Microtus pennsylvanicus*), 17 white-footed mice (*Peromyscus leucopus*), 17 house mice (*Mus musculus*), 4 short-tailed shrews (*Blarina brevicauda*), one weasel (*Mustela frenata*), and one jumping mouse (*Zapus hudsonius*). Tissues from only one of the 105 wild caught animals contained a demonstrable rickettsial agent; the guinea pigs inoculated with tissues from the other 104 animals remained afebrile, showed no signs of disease and failed to develop complement-fixing antibodies against spotted fever.

A strain of rickettsiae, subsequently identified as belonging to the spotted fever group, was isolated from the tissues of an apparently healthy *Microtus* (No. B14009) trapped on June 18, 1952 near Alexandria, Va. Three guinea pigs, each inoculated intraperitoneally with one ml of a suspension of brain, spleen and liver from this animal, developed fevers of 105°F on the fifth day following inoculation. One of the sick guinea pigs was killed on the third day of fever and a suspension of its spleen, liver and testes was inoculated intraperitoneally into normal guinea pigs. The *Microtus* agent was maintained through more than 15 passages in guinea pigs. The passage materials were bacteriologically sterile.

The characteristic disease induced in guinea pigs by the *Microtus* agent had an incubation period of 2 to 8 days and a febrile phase of 5 to 9 days duration but was not lethal. The agent produced no scrotal reactions in early passage guinea pigs, but in later passages some animals developed swelling and erythema of the scrotum. However, the necrosis and sloughing of superficial scrotal tissues, ears and foot pads characteristically produced in guinea pigs by the Bitterroot strain of *R. rickettsii* were not observed in animals infected with the *Microtus* agent.

Postmortem examination of infected guinea pigs revealed splenomegaly, inguinal lymphadenopathy and hyperemia of the testes and tunica vaginalis. Rickettsiae were extremely scarce in smears prepared from tissues of early passage guinea pigs, but were seen occasionally within the cytoplasm of cells in smears from scrapings of the tunica vaginalis of later passage animals.

The *Microtus* agent was lethal for chick embryos 4 to 6 days following inoculation, and bacilliform rickettsiae were seen in smears from the yolk sacs of infected eggs. Known infectious material failed to produce obvious disease in white mice during 2 serial passages in that host.

Convalescent sera from guinea pigs which had been infected with the *Microtus* agent fixed complement in the presence of specific Rocky Mountain spotted fever antigen at titers of from 1/40 to 1/160, but these same sera failed to fix complement in the presence of rickettsialpox specific antigen.

Guinea pigs recovered from infection with the *Microtus* agent resisted infection with the Azain and Bitterroot strains of *R. rickettsii.* Similarly, Azain-immune guinea pigs were resistant to overt infection with the *Microtus* agent. Results of the cross-immunity tests are given in Table I.

Discussion. The recovery of rickettsiae of the spotted fever group from a wild mammal is of special interest from the standpoint of the ecology of Rocky Mountain spotted fever in this country. Although naturally infected mammals have never been definitely demonstrated in the United States, strains of spotted fever have been isolated from wild-caught opossums (*Didelphis aurita, D. paraguayensis,* and *D. marsupialis*) in Brazil(2,3). Brazilian workers(3) have also reported dogs, rabbits (*Sylvilagus minensis*) and cavies (*Cavia aperea*) to be susceptible to experimental infection with spotted fever. However, since

TABLE I. Relation of *Microtus* Agent to *R. rickettsii*: Cross-Immunity Tests in Guinea Pigs.

Challenge strain	Guinea pig group	Results of challenge: Fever — No. G.P. with	Fever — Avg days duration	Scrotal reaction — No. G.P. with	Scrotal reaction — Intensity*	No. G.P. survived/No. tested
RMSF (Azain strain)	*Microtus* agent					
	Immune	0/2	0	0/2	0	2/2
	Normal	2/2	10	2/2	4+	2/2
RMSF (Bitterroot strain)	*Microtus* agent					
	Immune	0/2	0	0/2	0	2/2
	Normal	2/2	10	2/2	4+	1/2
Microtus agent	Azain immune	0/2	0	0/2	0	2/2
	Normal	2/2	8	0/2	0	2/2

* Scrotal reaction intensity: 1+ to 2+ = swelling and erythema; 3+ = swelling, erythema and adhesion of testicles; 4+ = necrosis and sloughing of scrotal skin plus swelling, erythema and adhesion of testicles.

quite different species of ticks and mammalian hosts are involved, the ecology of spotted fever in South America may differ greatly from that which exists in North America.

In the United States, Jellison(4) has suggested that the cottontail rabbit *Sylvilagus nuttallii,* may be an important reservoir of spotted fever. The distribution of this rabbit coincides roughly with that of the disease in the western United States, and wild cottontails are important hosts of the Rocky Mountain wood tick, *Dermacentor andersoni,* which is the principal vector of the disease in the West. Protection tests carried out by Parker(5) suggested the occurrence of spotted fever infections in wild rabbits and rodents. Complement-fixing antibodies against spotted fever rickettsiae have been demonstrated by Parker and his coworkers(6) in the sera of wild-caught cottontails and snowshoe rabbits (*Lepus americanus*) in Montana, and these authors also isolated strains of spotted fever from rabbit ticks, *Haemaphysalis leporis-palustris,* collected from cottontails in the same region. Jellison(4) and Parker *et al.* (7) have cited the probable isolation of the agent of spotted fever by Hassler and his coworkers from a pocket gopher (*Geomys breviceps dutcheri*) trapped in Oklahoma. Unfortunately, the agent isolated from the gopher was lost before its identity was established. Shepard and Topping(8) reported the presence of complement-fixing antibodies in the sera of dogs from several known endemic regions of the United States. Miller (9) found that experimentally infected foxes and raccoons showed no visible signs of disease, but they did respond by the formation of complement-fixing antibodies against *R. rickettsii.*

Seven of the *Microtus* used in this study, including the one positive animal, had one or more nymphs of *Dermacentor variabilis* attached to them when collected. This tick is the principal vector of spotted fever in the eastern half of the United States. While the adults of *variabilis* frequently attack man, this stage is more often found on dogs, livestock, deer and wild carnivores. The larval and nymphal stages parasitize smaller mammals, particularly rodents. All stages of this tick are able to transmit spotted fever, and the infection can be transferred from stage to stage and to successive generations through the eggs of infected female ticks(10). The eastern meadow mouse, *Microtus pennsylvanicus,* is one of the most common hosts of the immature stages of *D. variabilis* where the range of the tick and meadow mouse coincide(11). The distribution of *M. pennsylvanicus* and its subspecies, according to Hall and Cockrum(12), extends over most of Canada and the northern half of the United States from the Atlantic seaboard as far west as British Columbia and northeastern Washington, and it is found as far south as Georgia and northern New Mexico. Its distribution coincides to a great extent with that of *D. variabilis* in the eastern United States and extends into the range of *D. andersoni* in the Rocky Mountain region. Jellison(13) found the meadow mouse highly susceptible to ex-

perimental infection with spotted fever, and he was able to demonstrate tick-to-mouse-to-tick transfer of the infection in this host by the simultaneous feeding of infected nymphs and non-infected larvae of *D. andersoni* on the same animal. The present paper provides the first direct evidence of the occurrence of spotted fever infections in meadow mice in nature. However, it should be emphasized that the findings of this study do not necessarily give an indication of the actual prevalence of spotted fever infections among wild meadow mice, or for that matter, among the other species of mammals dealt with in this study. Furthermore, the role played by these mammals in the maintenance of spotted fever in nature remains to be determined.

Summary. The recovery of a strain of rickettsia belonging to the spotted fever group, presumably *Rickettsia rickettsii*, from the tissues of a wild meadow mouse, *Microtus pennsylvanicus*, is described. The evidence supporting its identification as *R. rickettsii* is presented and the implications of these findings are discussed.

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Skin Sterols IV. Distribution of Δ^7-Cholestenol.* (20951)

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Δ^7-cholestenol, 7-dehydrocholesterol, and certain other sterols react much more rapidly in the Liebermann-Burchard test than cholesterol itself, and this difference in rate has been made the basis for a determination of total "fast-acting" sterols(1,2). 7-dehydrocholesterol, which occurs in the skins of most species examined(3), can be measured spectrophotometrically and the concentration of Δ^7-cholestenol and related substances calculated by difference. Commercial cholesterol contains 0.62% of the Δ^7 sterol, and the results of a selenium test suggest that sterols from certain other sources may contain as much as 4.34%(4). Much larger amounts of Δ^7-cholestenol, 23-25% of the total sterols, have been isolated from rat skins(5).

Experimental. Twenty-five to 500 g of fresh skin (single skin or pooled samples) were extracted with acetone and the sterols isolated as the digitonide by procedures previously outlined(5). Dry digitonide containing 50-500 μg of sterol was placed in a standard Evelyn tube and dissolved by warming with 2 ml of glacial acetic acid. After cooling, 4.2 ml of reagent (freshly prepared mixture of 20 ml of redistilled acetic anhydride and one ml of H_2SO_4 cooled to room temperature) was added and the color read in an Evelyn colorimeter at 1.5 and 38 minutes.

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