In 1904, an impressive article was published by Wilson and Chowning in the first volume of The Journal of Infectious Diseases [1]. Their investigations in the Bitter-root Valley of western Montana had been stimulated by the high incidence of a severe disease, known locally as “spotted fever,” that had a high case-fatality rate, spring seasonality, and lack of communicability. It was truly an emerging infectious disease of unknown cause. Their valid observations included the first description of severe Rocky Mountain spotted fever: clinical features (onset, incubation period, symptoms, rash, and course), findings from 8 autopsies, geographic distribution on the western side of the Bitterroot River, and negative bacterial cultures. Most cases occurred between May 15 and June 15 among 20–40-year-old males, because of “increased exposure to infection through their occupation or pleasure taking them outdoors in the foothills and mountains in the spring of the year” [1]. Wilson and Chowning’s description of the presence of intraerythrocytic protozoa in patients and in Columbian ground squirrels, as well as their related hypothesis of transmission by tick bite, were broadly acclaimed and then subsequently rejected when the acknowledged experts—Stiles, Ashburn, and Craig—found no protozoa in the patients’ blood.

THE IMPACT OF RICKETTS’ 1906 RESEARCH

Howard T. Ricketts chose to answer the appeal from Montana for further scientific study of the problem. At age 33 he was well prepared, having graduated from Northwestern University School of Medicine in 1897 and trained in pathology at Rush Medical College, where his research focused on blastomycosis [2]. Before joining Ludvig Hektoen at The University of Chicago in 1902, Ricketts studied in Berlin, Vienna, and Paris for a year, enhancing his knowledge of both the concepts and laboratory methods of microbiology. The publication of his Infection, Immunity, and Serum Therapy [3] in 1906 established his credentials as a creative young scientist in infectious diseases. His goal was to address the muddled problem of Rocky Mountain spotted fever independently and with an open mind.

Funded by a small grant from the American Medical Association and by private funds, Ricketts arrived in Missoula, Montana, in late April 1906 and set up tents for his laboratory and housing, on the grounds of Northern Pacific Hospital [4]. His investigations during that year revealed most of the important principles related to rickettsiae (in particular, Rickettsia rickettsii) and to Rocky Mountain spotted fever. Ricketts had published 3 other reports [5–7] on different aspects of his research, before his article “Observations on the Virus and Means of Transmission of Rocky Mountain Spotted Fever” [8] was published in the Journal in 1907. These earlier articles, summarized in the introduction to the 1907 volume, described experimental transmission of the infection from the blood of patients to guinea pigs and monkeys, maintenance of the agent via serial animal passages alternating between monkeys and guinea pigs, and the ability of a female tick to acquire the infection by feeding on an experimentally infected guinea pig and to subsequently transmit the illness by feeding on another guinea pig. The latter phenomenon was simultaneously reported by King [9], with whom Ricketts had shared his ideas and materials [5–7, 9]. Ricketts also reported in those first 3 articles preliminary observations regarding the nonfilterability of the
The heating and drying conditions that result in the loss of viability of the organisms and elucidated the kinetics of the slow loss of viability of rickettsiae stored in an ice chest.

The footnote to table 1 in Ricketts’ 1907 paper describes one of his most important discoveries in 1906: the gross pathology of experimentally infected guinea pigs, including “hemorrhages into the skin of the external genitalia, and, in males, into the testicles and their coverings” (p. 144). For the first time there was a definitive, objective laboratory method for the diagnosis of Rocky Mountain spotted fever and for the detection of the presence of the etiologic agent in ecologic as well as clinical samples. Even before Flexnerian reforms in medical education, astute clinicians of the American West had noted that the disease was quite similar to typhus, but confusion abounded because its signs and symptoms were similar to those of other diseases. With Ricketts’ findings, Rocky Mountain spotted fever became a true disease, not a syndrome. The macroscopic changes in infected guinea pigs yielded a distinctive criterion for the diagnosis of the infection.

AN EPILOGUE: RICKETTS’ DISCOVERIES FROM 1907 TO 1910

Ricketts devoted the remainder of his life—a life that ended because of laboratory-acquired typhus fever in Mexico City in May 1910, only 4 years after he first set foot in western Montana [2]—to the study of what are now known as “rickettsioses.” Recognizing that the agent of Rocky Mountain spotted fever resided in nature, he devoted much effort to elucidating its niche, with the aim of developing an approach to control the disease. He determined that vector ticks transmit the agent via their eggs to the next generation, and he documented the maintenance of the agent as a larva molts, after its blood meal, to become a nymph and, subsequently, as the nymph molts, after its blood meal, to become an adult male or female tick. He recognized early and proved definitively that transmission was biological, not merely mechanical. Part of the transmission-by-tick hypothesis proposed by Wilson and Chowning was based on the scientific dogma of the time—that arthropods biologically transmit only parasites, examples being the transmission
of the Texas cattle fever agent by ticks and of the filariasis and malaria agents by mosquitoes; in contrast, arthropods were thought to transmit bacteria only mechanically. Stiles rejected the protozoal etiology equally for its heretical transmission-by-tick hypothesis and for the absence of protozoa in blood smears. Ricketts’ experimental data, along with others’ later studies of plague, typhus, tularemia, borrelioses, and bartonelloses, contributed to the downfall of this dogma.

During his studies of *R. rickettsii* in ticks, Ricketts observed small bacilli that were microscopically similar to what he had observed in infected blood. Using these tick-derived organisms, he developed an agglutination test that demonstrated antibodies in convalescent sera with reciprocal titers as high as 1:320. Nonimmune sera did not agglutinate the tick-associated bacteria—further evidence for a bacterial etiology and a tick host.

Ricketts’ later discovery of tick-associated bacteria that were similarly agglutinated by immune sera but that did not cause infection in guinea pigs puzzled him. Of course, he was unaware of the existence of genetically related but nonpathogenic *rickettsiae*, such as *R. peacockii* and *R. montanensis*, that share antigens with *R. rickettsii*. Indeed, the high prevalence of *R. peacockii* on the eastern side of the Bitterroot River competitively excluded the establishment of transovarian transmission of *R. rickettsii* by ticks, explaining the data assembled by Wilson and Chowning that showed that Rocky Mountain spotted fever occurred only on the western side of the river [17, 18]. Ricketts’ definitive proof of the importance of ticks in the maintenance of *R. rickettsii* was his demonstration that infected ticks existed in nature. The low percentage of ticks that carry *R. rickettsii* explained the fact that a large number of tick bites resulted in a relatively small number of infections. Only much later was it recognized that *R. rickettsii* is also mildly virulent for ticks, the most likely explanation for the low proportion of infected ticks in nature [19].

Ricketts also investigated the possibility that a mammalian host might play a role in the maintenance of *R. rickettsii* in nature [4]. Columbian ground squirrels, chipmunks, and woodchucks were shown to be susceptible to an *R. rickettsii* bacteremia that, while relatively short in duration, was present at levels high enough to infect feeding ticks. Efforts to control the disease by the reduction of the populations of ticks and mammalian hosts have been difficult and have potentially harmful effects.

The final aspect of Rocky Mountain spotted fever to which Ricketts contributed was immunology. He demonstrated passive serum prophylaxis of experimental disease in guinea pigs, but, of 9 human patients treated with hyperimmune horse serum, 6 died [2, 20]. We now know, of course, that treatment with antibodies given late in the course of infection has little effect on the outcome [21]. Ricketts also utilized serovaccination in guinea pigs; the appropriate quantity of antibody modified the effect that an appropriate dose of *R. rickettsii* had on the stimulation of immunity. The tricky problem was establishing conditions that were uniformly effective and never fatal.

**Ricketts’ Legacy and Unresolved Problems of Rickettsiology**

Ricketts’ investigations were of long-lasting significance. The impact of his discovery of the first vector-borne obligately intracellular bacterium is emphasized in the names that were eventually applied to the genus, “*Rickettsia*”; the species, “*rickettsii*”; vector-borne obligately intracellular bacteria, “rickettsiae”; and a branch of microbiology, “rickettsiology.” This well-educated, dedicated, hardworking man with a microscope; some guinea pigs, monkeys, and ticks; and only a few assistants began the demonstration that uncultivated, obligately intracellular bacteria cause many diseases. Furthermore, Ricketts did so under conditions rather foreign to scientists today. In 1906, Rocky Mountain spotted fever was a terrifying emerging infectious disease. Many workers, particularly in the preantibiotic era, died of laboratory-acquired *R. rickettsii* infection, and Ricketts died of infection from a related dangerous pathogen, *R. prowazekii*. It was equivalent to performing Ebola-virus research without protective laminar-flow safety cabinets, biosafety level 3 and 4 laboratories, or such tools as cell culture, polymerase chain reaction, DNA sequencing, and electron microscopy. Yet, even as recently as the prolonged search for the cause of Legionnaire’s disease, it was the guinea pig inoculation of infected human tissues, performed by a rickettsiologist, Joseph McDade, that first recovered the disease’s etiologic agent, *Legionella pneumophila* [22]. It is curious that the many rickettsial agents discovered recently as the causes of emerging infectious diseases—agents such as *R. japonica*, *R. honei*, *R. felis*, *R. africae*, *Ehrlichia chaffeensis*, *E. ewingii*, *E. muris*, and *Anaplasma phagocytophilum*—have not attracted as much attention and scientific effort as have the many newly discovered pathogenic viruses; nor has antibiotic-resistant *R. prowazekii* received as much concern as have many less-pathogenic agents that have potential for dispersal by bioterrorists [23–26].

The list of unanswered questions regarding rickettsiae is longer today than it was in 1910. In the realm of how rickettsiae actually cause disease, significant unanswered questions include the following:

- What are the adhesins by which rickettsiae first attach to endothelial cells?
- What molecular mechanism(s) mediate rickettsial escape from the phagosome into the cytosol?
- What is the importance, in animal models and in human infections, of the *R. rickettsii* injury to endothelial cells that is caused by the stimulation of endothelial-cell production of reactive-oxygen species that has been characterized in vitro?
• What other rickettsia-mediated mechanisms of cell and tissue injury, including the activities of rickettsial proteases and phospholipases, occur in rickettsial diseases?
• What is the role that host-mediated mechanisms, such as the activities of cytokines and cytotoxic T lymphocytes, play in the pathogenesis of tissue injury?
• What are the molecular and cellular mechanisms that cause increased vascular permeability, the hallmark pathophysiologic effect of rickettsioses?

To develop an effective vaccine against rickettsiae, the following must be determined:

• The role and mechanisms of innate immunity to rickettsiae, including the identification of the initially infected cells (i.e., macrophages, dendritic cells, endothelium, or dermal fibroblasts) and of the most effective antigen-presenting cells;
• The effects of tick inoculation (i.e., immunomodulatory effects of tick saliva, the prolonged time course of inoculation, and the mechanism of rickettsial reactivation during tick feeding);
• The course and route of spread from the site of tick inoculation (i.e., rapid vs. local growth and slow spread; lymphatic vs. blood vessels);
• The mechanisms responsible for perivascular emigration of immune CD4 and CD8 T lymphocytes and macrophages at sites of endothelial infection;
• Whether nitric oxide–, reactive-oxygen species–, or tryptophan degradation–dependent antirickettsial mechanisms are active and important in vivo in human rickettsioses;
• The immunodominant rickettsial antigens responsible for (1) stimulating CD4 and CD8 T lymphocytes to secrete protective cytokines, (2) stimulating protective antibodies other than anti–outer-membrane proteins A and B, and (3) CD8 cytotoxic T lymphocyte activity, which is associated with the clearance of rickettsiae from the endothelium;
• The mechanism and effects of rickettsial infection–associated transient immunosuppression;
• The location and mechanism of R. prowazekii survival as a latent infection—and the factors and mechanisms that reactivate latent typhus, leading to Brill-Zinsser disease.

Just as important is the list of yet-to-be-developed laboratory tools—for example, a genetic system for knocking out or substituting rickettsial genes so that effective vaccines can be developed and virulence factors can be elucidated, and an affordable, available, ultrasensitive, specific, timely, point-of-care diagnostic test for rickettsial diseases in the acute stage.

It is a tragedy that Ricketts died in 1910. He would not have solved all of these problems had he lived another 25 or 30 years, but he would have advanced our knowledge of rickettsial and other infectious diseases faster, and he would have stimulated and educated one or more generations of new infectious-diseases scientists who would have changed the world. Perhaps, as a result, someone would have noted that rickettsial diseases are not eliminated by the use of doxycycline. Optimistically, the availability of rickettsial genome-sequence information would have been exploited more effectively to unravel pathogenesis and immunity, rather than to detail the minutiae of phylogeny. Rickettsiology today needs an infusion of new blood, particularly of more scientists who understand, as Ricketts did, infectious diseases and microbial pathogenesis.

References
6. Ricketts HT. The transmission of Rocky Mountain spotted fever by the bite of the wood-tick (Dermacentor occidentalis). JAMA 1906; 47:358.
7. Ricketts HT. The role of the wood-tick (Dermacentor occidentalis) in Rocky Mountain spotted fever, and the susceptibility of local animals to this disease—a preliminary report. JAMA 1907; 49:24–7.
18. Niebylski ML, Schrumpf ME, Burgdorfer W, Fischer ER, Gage KL,


