Rocky Mountain Spotted Fever: a Disease in Need of Microbiological Concern

DAVID H. WALKER

Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77550

INTRODUCTION	227
HISTORY OF RMSF	227
ETIOLOGIC AGENT	228
ECOLOGIC AND EPIDEMIOLOGY	230
PATHOGENESIS	230
CLINICAL MANIFESTATIONS	232
CLINICAL DIAGNOSIS	233
LABORATORY DIAGNOSIS	233
Serologic Diagnosis	234
Visualization of Organisms in Tissues	235
FUTURE PROSPECTS	236
ACKNOWLEDGMENTS	236
LITERATURE CITED	236

INTRODUCTION

Rocky Mountain spotted fever (RMSF), one of the most severe of all infectious diseases, was first recognized on the frontier of the American West (80). Brilliant scientists of the early 1900s advanced our general concepts regarding the microbial ecology, pathogenesis, and taxonomy of RMSF during their investigations of the disease (108). Efforts to elucidate the rickettsioses have been diverted by wars, epidemics, and other newly recognized infections (161). The development of antimicrobial agents that are effective when given early in the course of infection and the cyclic waning of disease incidence, as occurred concurrently in the late 1940s, led many to conclude incorrectly that the problem had finally been solved. Resurgence in the incidence, difficulty of clinical diagnosis, defined populations at higher risk of a fatal outcome, and increased general use of antimicrobial agents that lack antirickettsial activity are persistent factors leading to misdiagnosis and death. Failure of vaccines to confer protective immunity and the lack of a generally available laboratory diagnostic test during the acute stage of illness provide overwhelming evidence that the old problems of prevention and diagnosis of RMSF still need attention (14, 34, 37, 39, 40, 53, 54, 63, 65, 67, 87, 136, 139, 149, 151, 153, 156, 164).

HISTORY OF RMSF

Efficient transovarian transmission of *Rickettsia rickettsii* (the etiologic agent of RMSF) from one generation of ticks to the next implies a long evolutionary period to arrive at the current state of adaptation and, thus, suggests that the organism was in the Americas prior to the arrival of humans (21-24). The first illness attributed later to RMSF occurred in 1873 among settlers of the Bitterroot Valley of western Montana. The first clinical description of the rickettsiosis, "Some Observations on the So-called Spotted Fever of Idaho," was published in 1899 (80).

The most important early steps in the determination of the etiology, pathogenesis, and epidemiology of RMSF were taken by the pathologists Louis B. Wilson and William M. Chowning from the Minnesota State Board of Health and the

University of Minnesota, Howard T. Ricketts from the University of Chicago, and S. Burt Wolbach from Harvard University (50). Wilson and Chowning made astute scientific contributions. They described the geographic and seasonal distribution, the age and sex-specific incidence and mortality rates, clinical signs and symptoms, and the pathologic lesions (167). They also documented the absence of a conventional bacterial etiology and predicted that the disease had an infectious etiology and a tick vector. Unfortunately, these important observations were largely overshadowed by their subsequent mistake in attributing the disease to an erythrocytic protozoan etiology, in "Studies in *Pyroplasmoides hominis* ('Spotted Fever' or 'Tick Fever' of the Rocky Mountains)" (167). Between 1906 and 1909, Ricketts and co-workers (114, 115, 161) demonstrated that RMSF was caused by infectious organisms that were present in the patient's blood, could be transmitted to guinea pigs and monkeys, were similar to bacteria in being retained by a filter, conferred immunity on animals that survived the infection, and stimulated antibodies that neutralized infectivity and agglutinated organisms. The tick was also incriminated as a reservoir and vector at this time. Transovarian transmission from one generation of ticks to the next offered an explanation for the persistence of the organism in nature. Ricketts also reported that the Rocky Mountain wood tick could transmit the infectious agent from an infected guinea pig to an uninfected guinea pig. In 1908, McCalla reported an experiment involving human volunteers that was conducted 3 years earlier, before the first studies by Ricketts (81). A tick removed from a patient with RMSF was allowed to feed sequentially on two healthy volunteers, who subsequently developed classic RMSF. These experiments clearly documented that a tick bite could transmit the infection to guinea pigs and humans, but they did not show that adult ticks acquire the organism from one host and then transmit it to the next. Because a longer period is required for generalized infection to develop in the tick, during which rickettsiae spread from the gut of the tick to the salivary glands, rickettsial infection transmitted by adult ticks is actually acquired by ticks at an early stage of development, usually as an ovum. These details, as well as the observation that

many rickettsia-infected ticks do not cause disease, were elucidated only after years of studies of ticks in the laboratory and the discovery that nonpathogenic rickettsial species occur in nature. Between 1916 and 1919, Wolbach first convincingly visualized the etiologic agent by light microscopy and demonstrated its presence in the ova and all developmental stages of ticks. He also demonstrated the organisms in damaged vascular endothelium and smooth muscle of humans and experimentally infected guinea pigs, monkeys, and rabbits (50, 177, 178).

Attempts to develop a vaccine have marched in step with the ability to propagate the fastidious, obligate, intracellular bacterium. In 1923, a vaccine containing a heated mixture of *R. rickettsii*-infected material from guinea pigs and immune sera from rabbits was tested by Hideyo Noguchi of the Rockefeller Institute (92, 108). The foreign antigens in the vaccine caused immune complex disease, and the vaccine did not protect against RMSF. Shortly thereafter, R. R. Spencer developed a vaccine, using phenol-killed rickettsiae propagated in vivo in infected ticks (98, 131). Field studies in the late 1920s showed a reduction in the case fatality rate among infected vaccinated persons in the Bitterroot Valley and prevention of illness in sheepherders in southern Idaho, where a naturally milder form of the disease was prevalent at that time. A major scientific breakthrough occurred in 1938, when Herald Cox discovered that large quantities of rickettsiae could be cultivated in the yolk sac of embryonated hen eggs (36). It enabled acquisition of sufficient rickettsiae for the easier preparation of a Formalin-killed rickettsial vaccine, as well as for studies leading to the understanding of rickettsiae as organisms. Ultimately, killed rickettsial vaccines derived from ticks, embryonated eggs, and, most recently, cell culture all failed to protect vaccinated volunteers challenged with virulent *R. rickettsii* (34, 40). Currently, no vaccine is available for the prevention of RMSF.

Entomologists have played an important role in the clarification of the natural history of *R. rickettsii*, beginning with the collaboration of Robert Cooley and Ricketts (108). The achievements of R. R. Parker and Willy Burgdorfer at the Rocky Mountain Laboratories in Hamilton, Mont., have spanned most of the years between 1914 and the present. Parker discovered that the tick's blood meal reactivates rickettsial virulence (129). Burgdorfer analyzed the rickettsia-tick relationship; he and co-workers discovered several nonpathogenic rickettsiae and demonstrated that they interfere with establishment of pathogenic *R. rickettsii* in ticks (23, 27, 28, 30).

ETIOLOGIC AGENT

R. rickettsii, the causative agent of RMSF, is a small (0.2 to 0.5 by 0.3 to 2.0 μm), obligate, intracellular bacterium (162). Ultrastructurally, these organisms have typical prokaryotic cytoplasm containing ribosomes and indistinct strands of deoxyribonucleic acid (DNA) in an amorphous cytosol that is surrounded by a plasma membrane. The outer envelope or cell wall resembles that of other gram-negative bacteria. Within the genus *Rickettsia*, the ultrastructural appearance of the cell wall of *R. rickettsii* and other members of the spotted fever and typhus groups is distinguishable from that of *R. tsutsugamushi*, the etiologic agent of scrub typhus (125). This observation is but one of many indicating that *R. tsutsugamushi* and *R. rickettsii* are very different and share features possibly only as result of convergent evolution. A peptidoglycan layer is assumed to exist, but has not been identified in the periplasmic space. An indistinct layer,

often referred to as the microcapsular layer, is present on the outer surface of the cell wall. An electron-lucent zone separates this layer from the host cytosol. This zone is believed to represent a slime layer that can be stained with ruthenium red and methenamine silver (107). It is observed extracellularly as a distinct structure only when stabilized with antibodies (127). In contrast to the other genera of obligate intracellular bacteria that are pathogenic for humans (*Chlamydia*, *Coxiella*, and *Ehrlichia*), organisms of the genus *Rickettsia* are not surrounded by a host cell membrane, but rather reside directly in the cytosol or nucleoplasm of the host.

R. rickettsii is a member of the antigenically defined spotted fever group of the genus (103, 162). Other named species of the spotted fever group are *R. conorii* (boutonneuse fever), *R. sibirica* (North Asian tick typhus), *R. akari* (rickettsialpox), *R. australis* (Queensland tick typhus), and the presumably nonpathogenic rickettsiae *R. parkeri*, *R. montana*, *R. rhipicephali*, *R. helvetica*, and *R. heilongjiangi* (11, 30, 77, 96, 100). In addition, a unique related human pathogen has been discovered recently in Japan (134). There are at least five unnamed, apparently nonpathogenic rickettsial species of the spotted fever group in ticks in the United States and two unnamed species from ticks collected in Thailand and Pakistan (27, 28, 64, 103, 116). Organisms isolated from ticks and traditionally designated as *R. conorii* seem to possess previously unrecognized antigenic and genetic diversity (*R. Regnery*, Abstr. Congr. Int. Coll. Rickettsiologists 1987, p. 90, Palermo, Italy; X. J. Yu, D. H. Walker, and T. R. Jerrells, unpublished data).

Although antigenically distinguishable from one another, rickettsiae of the spotted fever and typhus groups form a genetically interrelated complex. They have substantial phenotypic differences from the scrub typhus group. The genome of *R. rickettsii* contains 1.3×10^9 daltons (Da) of DNA. The guanine-plus-cytosine content of *R. rickettsii* and other spotted fever group rickettsiae is 32 to 33 mol% compared with 29 to 30 mol% for typhus group rickettsiae (133). DNA relatedness as determined by DNA-DNA hybridizations between *R. rickettsii* and other species is remarkably close: 91 to 94% for *R. conorii*, 70 to 74% for *R. sibirica*, 73% for *R. montana*, 53% for *R. australis*, 46% for *R. akari*, 47% for *R. prowazekii*, 42% for *R. typhi*, and 37 to 47% for *R. canada* (89, 90; W. F. Myers and C. L. Wisseman, Jr., Abstr. Third Natl. Conf. Am. Soc. Rickettsiol. Rickettsial Dis. 1982, abstr. no. 21). The last organism shares features of both the typhus and spotted fever groups, emphasizing the close relatedness of these two groups. Another related organism, *R. bellii*, is highly prevalent in ticks in the United States and does not fit well into either group (102).

Among organisms bearing the designation *R. rickettsii*, there seem to be at least two subspecies (3, 4, 7, 97). The first subspecies is represented by isolates obtained from humans and some of the isolates from ticks that are known to transmit RMSF. A unique subspecies, *R. rickettsii* strain HLP, was originally found in the rabbit tick *Haemaphysalis leporispalustris*, which does not feed on humans and, thus, would not transmit this organism to humans (97, 101). In the early 1950s, quantified doses of virulent *R. rickettsii* and other indigenous spotted fever group rickettsiae of unidentified species were propagated in embryonated eggs and inoculated into guinea pigs. The strains were then placed in categories on the basis of virulence for guinea pigs (109). These data as well as the high case fatality rate (75%) in the Bitterroot Valley of western Montana in the early 1900s (167)

led some investigators to suggest that strains of *R. rickettsii* recovered from patients in the western United States are more virulent than those from patients in the eastern United States (23). Indeed, Anacker et al. identified two strains of *R. rickettsii* isolated from humans in western Montana that caused greater mortality, more severe scrotal lesions, and longer duration of fever in experimentally infected guinea pigs than two strains isolated from humans in North Carolina. The more pathogenic strains from Montana are distinguished from the North Carolina strains by unique heat-labile epitopes on the 120-kDa surface protein (3). It should be emphasized, however, that the electrophoretic patterns of the proteins of human isolates of *R. rickettsii* separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis are indistinguishable. Moreover, some monoclonal antibodies react with both Eastern and Western strains of *R. rickettsii*, but not with other spotted fever group rickettsiae including *R. rickettsii* HLP (4). Analysis of the diversity of nucleotide sequences in a collection of isolates of *R. rickettsii* demonstrated minimal differences (P. Fuerst, Abstr. Seventh Natl. Conf. Am. Soc. Rickettsiol. Rickettsial Dis. 1988, abstr. no. 18, p. 5). The genome of *R. rickettsii* strains that cause RMSF is concluded to have been highly conserved.

Although also closely related to virulent *R. rickettsii*, strain HLP is phenotypically and ecologically distinct and deserves separation from the other strains classified as *R. rickettsii* (3). HLP strains have never been demonstrated to cause human illness, having been recovered only from ticks. HLP strains cause a relatively mild, nonfatal, febrile illness when inoculated into guinea pigs. Heat-labile epitopes on each of the major surface proteins (120 and 155 kDa) serve to distinguish HLP strains from other strains of *R. rickettsii*, and HLP strains also possess distinctive electrophoretic bands by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (3-5). It would be intriguing to know whether HLP strains are capable of ever infecting humans, what signs and symptoms (if any) would result, and whether subsequent immunity to virulent *R. rickettsii* would ensue.

R. rickettsii contains numerous proteins that are detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (94, 99). Antibodies to most of them are present in convalescent sera from humans and animals (2, 42, 166). There is evidence for surface exposure of seven polypeptides. In addition, the cell wall contains lipopolysaccharide (LPS) that is also exposed on the surface (2, 138). Two surface proteins, generally designated as 120 and 155 kDa, may be considered as the immunodominant antigens because antibodies to these proteins appear early and hybridomas secrete monoclonal antibodies predominantly to these surface proteins and LPS (2, 71, 75, 138). Most monoclonal antibodies to heat-labile epitopes of the 120- and 155-kDa polypeptides neutralize the ability of the rickettsiae to cause febrile disease in guinea pigs and to cause toxic death in mice inoculated intravenously with lethal doses of bacteria. In contrast, monoclonal antibodies to heat-stable epitopes of the 155-kDa polypeptide and to LPS do not neutralize mouse toxicity. The electrophoretic mobilities of the two major surface polypeptides are heat modifiable, having faster mobility in the native state. The genes for both the 155- and 120-kDa polypeptides have been cloned into *Escherichia coli*, and expression of the antigens has been achieved (85; G. A. McDonald, Abstr. Seventh Natl. Conf. Am. Soc. Rickettsiol. Rickettsial Dis. 1988, abstr. no. 23, p. 7). In preliminary studies, immunization of guinea pigs and mice with recombinant *E. coli* resulted in reduced morbidity and

mortality upon infectious challenge and intravenous toxicity challenge, respectively (85, 86; G. A. McDonald and R. L. Anacker, Abstr. Sixth Natl. Conf. Am. Soc. Rickettsiol. Rickettsial Dis. 1986, abstr. no. 9, p. 9).

The most elusive component of *R. rickettsii* is the slime layer. Although visualized by electron microscopy, it has never been purified or characterized. It is rapidly lost during the process of separation of rickettsiae from the host cell components. The slime layer has been visualized with polyclonal antisera by electron microscopy (127). Study of the closely related organism, *R. conorii*, revealed that polyclonal antiserum to T-independent antigens stabilized this structure, albeit less effectively than sera containing antibodies to T-dependent rickettsial antigens (43). Monoclonal antibodies to LPS, the immunodominant T-independent antigen, and monoclonal antibodies to the surface proteins did not stabilize the slime layer (43). These results suggest that, if the slime layer exists, it is probably more like an extracellular capsule than an accumulation of sloughed LPS or surface proteins. The location of this putative structure around the organism and a report that it increases in size when the tick is feeding, a time when virulence is increased, indicates that it should be an important concern for microbiological research (55).

The microbial physiology of *R. rickettsii* has not been studied extensively (10). As with all obligate intracellular organisms, determination of the metabolism of the parasite is difficult. In its natural intracellular location, the host metabolic pathways compound the problem. Outside of the cell it is difficult to be sure that the rickettsia is healthy and capable of its intracellular functions. Typhus group rickettsiae can be cultivated in greater quantity and separated from host cell components more efficiently than *R. rickettsii*, yielding much greater quantities of organisms for study (95). Thus, it is not surprising that most studies of rickettsial metabolic pathways have utilized *R. prowazekii* and *R. typhi* rather than *R. rickettsii* (15-19). Given the known differences among these two groups of rickettsiae, it would be wise to be cautious in drawing conclusions based on extrapolations of data from one species to another. Typhus rickettsiae possess many enzymes of the tricarboxylic acid cycle and nucleotide metabolism as well as active transport systems for adenosine diphosphate, monophosphate, and triphosphate, uridine diphosphoglucose, L-lysine, L-proline, and potassium (10, 128, 168, 170). Data on these metabolic activities are lacking for *R. rickettsii*, which does, however, metabolize glutamate, glutamine, pyruvate, and α -ketoglutarate (10, 113, 163). *R. rickettsii* synthesizes proteins in the absence of protein synthesis by the host cell (42). It is probable that availability of certain amino acids from host cell pools are limiting factors in rickettsial growth (9). The most important principle to consider regarding metabolism and physiology is that *R. rickettsii* has become highly adapted to intracellular life through eons of evolution. It thrives in the presence of high concentrations of potassium and protein, a low concentration of glucose, and the availability of intracellular constituents such as adenosine triphosphate. Far from being a defective life form, *R. rickettsii* has evolved mechanisms for survival in an unusual ecologic niche, the tick cell cytosol.

Rickettsiae must enter the host cell, proliferate, and escape in order to infect another host cell. It appears that rickettsiae attach to the plasma membrane of the endothelial cell of the host, induce the cell to phagocytize the rickettsiae, escape from the phagosome into the cytosol, and propagate by binary fission (141, 158, 159). *R. rickettsii* escapes from the host cell continuously by exit at the end of

long host-cell filopodia (141, 174). In contrast, typhus rickettsiae are released simultaneously when the host cell bursts (175). In fact, neither the rickettsial attachment mechanism nor the host cell receptor has been identified. Extrapolations from typhus rickettsial models (including one of in vitro hemolysis, which does not exist for spotted fever group rickettsiae) suggest the following hypotheses: (i) *R. rickettsii* may attach to a cholesterol-containing receptor on the host cell membrane; (ii) rickettsial energy derived from metabolism of glutamate to yield adenosine triphosphate and a functional host cell are required for induction of phagocytosis by the nonprofessional phagocytic cell; and (iii) phospholipase A activity, presumably of rickettsial origin, plays a role in the transit across the host cell membrane and escape from the phagosome (10, 110, 169). Reduction in cell injury by *R. rickettsii* in vitro (i.e., plaque formation) in the presence of compounds reported to inhibit phospholipase A or bind to cholesterol-containing receptors offers some support for hypotheses i and iii, although the conclusive experiments have not been conducted and the rationale may not apply to spotted fever group rickettsiae (146). That there is not a single protein of *R. rickettsii* to which a function can be ascribed emphasizes the need for microbiologic investigation of this organism.

ECOLOGY AND EPIDEMIOLOGY

R. rickettsii resides principally in various species of ticks, which are considered the reservoirs or natural hosts (23, 82). In the Rocky Mountain region, *R. rickettsii* is found in the wood tick *Dermacentor andersoni*. To the east of the range of this tick from the Great Plains to the Atlantic coast and in areas of the West coast, *R. rickettsii* occurs in *D. variabilis*, the common American dog tick (22). Other tick reservoirs are found in Mexico (*Rhipicephalus sanguineus*) and in Central and South America (*Amblyomma cajennense*). It is unclear whether *Amblyomma americanum* is a vector of RMSF.

The rickettsiae seem to be highly adapted to their tick hosts, which maintain the organisms efficiently both as they molt, passing the organisms to the next stage, and as they produce rickettsia-infected eggs, passing the organisms to the next generation (21–24). Transovarian transmission is the major mechanism for the maintenance of *R. rickettsii* in nature. There is a paucity of evidence for transmission from vertebrate hosts to ticks, yet the consensus opinion favors low-level horizontal transmission from ticks to mammals to new tick hosts (23, 82, 93). In the laboratory, after numerous generations of transovarian transmission, some ticks develop a massive increase in rickettsial numbers, which may result in decreased fecundity and death (23). Efficient transovarian transmission could theoretically maintain *R. rickettsii* forever, although even a small annual decrement in infected ticks would eventually result in extinction of the rickettsiae. Therefore, the evidence provided by the rare instances of isolation of *R. rickettsii* from wild mammals (29, 122) and the experimental documentation that rickettsiae do, albeit inconsistently, infect uninfected ticks during feeding indicate that a low level of initiation of newly infected tick lines does occur (25, 26; K. Gage, C. E. Hopla, and W. Burgdorfer, Abstr. Seventh Natl. Conf. Am. Soc. Rickettsiol. Rickettsial Dis. 1988, abstr. no. 35, p. 12). To put this equation into perspective, one must realize that, with the concession that there is local variability, the vast majority of vector species ticks do not contain any rickettsiae (23, 44, 101). Approximately 4% of *D. variabilis* ticks in many

locales contain spotted fever group rickettsiae, nearly all of which are presumably nonpathogenic (e.g., *R. montana* and *R. rhipicephali*). In large surveys, the prevalence of tick infection with the pathogenic species, *R. rickettsii*, is less than one per 1,000 ticks. Thus, the portion of the ecologic niche actually occupied by *R. rickettsii* is small. *R. rickettsii* apparently is not very successful at infecting a large proportion of ticks. If this supposition is true, humans are fortunate, for otherwise the incidence of RMSF could be much higher. On the other hand, nonpathogenic spotted fever rickettsial species seem to be able to exclude *R. rickettsii* from becoming established in ticks containing these nonpathogenic rickettsiae (23, 27). A nonpathogenic spotted fever group rickettsia dubbed the "East side agent" because of its high prevalence on the eastern side of the Bitterroot Valley prevents *R. rickettsii* from infecting the ovaries of *D. andersoni*. This form of interference explains why RMSF has always been confined to the area of the valley to the west of the Bitterroot River. The dynamic factors that exert changes in the numbers of ticks in nature and determine the proportion infected by *R. rickettsii* are largely unknown.

Following the recognition of this disease, great cyclic fluctuations in the incidence of RMSF have occurred over a period of decades (31, 53, 132). A dramatic increase in one region, such as occurred in the South Atlantic states during the 1970s, may accompany stable or decreasing incidence in other areas. The incidence in some parts of the Rocky Mountain region during the 1920s and 1930s was >50 cases per 100,000 population. Since the mid-1940s, the incidence in the Rocky Mountains has fallen to such extremely low levels that the name of the disease has virtually become a misnomer. Throughout the entire country in 1959, only 199 cases were reported to the Centers for Disease Control (CDC). After a steady steep rise in incidence, the highest number of cases, 1,192, was reported to the CDC in 1981. Subsequently, a decline has occurred, with 838 cases in 1984, 700 in 1985, and 592 in 1987 (31). In recent years the highest incidences have been in Oklahoma, North Carolina, and South Carolina. High incidences have also been reported in various years in Maryland, Virginia, Georgia, Tennessee, Ohio, Missouri, Arkansas, Texas, and Kansas. It is important, however, to realize that cases of RMSF have been reported in nearly every state. Endemic foci of RMSF are recognized in New York City and on Cape Cod and Long Island (57, 119, 135). The remainder of the descriptive epidemiology conforms to the seasonal activity of the vector ticks and their encounters with humans. Most cases occur between May and September, but though relatively rare, RMSF cases are reported in the winter months. The disease has a high incidence in children who play out-of-doors and have an affinity for dogs, which are frequently parasitized by *D. variabilis*. Nevertheless, since tick bites often are painless, many persons with RMSF are not aware of the event and do not recall it when questioned by their physicians.

PATHOGENESIS

The series of steps leading to the disease state begins with attachment of the adult tick to human skin. The vector tick species which transmit RMSF have three stages in their development (larva, nymph, and adult). The ticks consume only three blood meals during a lifetime: just before molting from larva to nymph, again before molting from nymph to adult, and during copulation as adults before the female lays eggs (22, 23). Only the adult stage of the tick feeds on humans; the larvae and nymphs of *D. variabilis* and *D.*

andersoni feed on small mammals. While the tick is attached to warm skin and imbibes a rich blood meal, the rickettsiae undergo reactivation from a dormant avirulent state to a highly pathogenic one (129, 130). Exposure to the thermal or nutritional factors or both for 24 to 48 h is required for reactivation of rickettsial pathogenicity. Furthermore, a variable period of time is also required for release of rickettsiae from the tick salivary glands and their inoculation into the human skin. This interval offers the best opportunity for prevention of RMSF by removal of the tick before the inoculation of rickettsiae. From the skin, the portal of entry, rickettsiae spread via the lymphatics and bloodstream to all parts of the body, including the skin, brain, lungs, kidneys, heart, liver, spleen, pancreas, and gastrointestinal tract (1, 63, 76, 111, 145, 151, 153, 154, 179). In each site the rickettsiae attach to, enter, and proliferate within endothelial cells. Subsequently, they are released from the endothelium, spread directly to adjacent endothelial cells, or invade deeper into the wall of the blood vessel to infect vascular smooth-muscle cells. *R. rickettsii* is not particularly well adapted to growth in human endothelium. Infection of this target cell occurs more as an accidental outcome of the encounter of the obligate intracellular pathogen with endothelium because of the hematogenous route of dissemination than because of any receptor-mediated tropism (147).

Evolution of the ability to parasitize human endothelium is of no benefit to the rickettsiae, which derive no profit from causing RMSF. The rickettsiae do not survive the interaction with humans, and they are not transmitted from human to other hosts. It is embarrassing to admit that we do not understand exactly how rickettsiae make us sick. Pathogenic mechanisms are understood for only a minority of infectious diseases, mainly those caused by exotoxins. The knowledge of most infectious diseases consists of a morass of microbiologic and clinical observations that have not been synthesized into a logical order. RMSF falls into this category.

An important question to be resolved is whether the rickettsia possesses functional activities that damage the host directly or whether the host response to rickettsial infection accounts for the clinical signs, symptoms, and tissue lesions (137). *R. rickettsii* kills heavily parasitized cells directly in vitro, thus demonstrating the rickettsial ability to exert its pathogenic mechanisms in the absence of the immune, inflammatory, and coagulation mechanisms of the host (141, 147, 148). A substantial body of experimental and human clinicopathologic data indicates that there is no important immunopathologic component to RMSF and that the immune system, particularly T lymphocytes and gamma interferon, is indeed crucial to the recovery of the host (20, 74, 150). In spite of the overall benefit of the immune response for the host, experimental in vitro data support the hypothesis that cytotoxic T lymphocytes and gamma interferon have the capacity to cause lysis of rickettsia-infected cells (117, 176). Thus, the suspicion persists that immunopathologic effects might occur during rickettsial infection and be overshadowed by the more significant beneficial effects of immunity.

Inflammatory mediators may also play a role in the genesis of some of the pathophysiologic effects in RMSF. The kallikrein-kinin system is activated (180); however, plasma levels of prostacyclin, thromboxane, and leukotrienes have not been reported in RMSF. Whether any of these inflammatory mediators actually contribute to the vascular pathophysiology in RMSF remains to be determined. In contrast, the intrinsic and extrinsic pathways of coagulation, platelets, and the fibrinolytic system are activated in many patients

with RMSF (112). The effect of these coagulation mechanisms in RMSF has often been erroneously referred to as disseminated intravascular coagulation. In RMSF, platelets and coagulation factors may be consumed in the foci where severe rickettsial infection has breached the integrity of the blood vessel wall. Thrombocytopenia occurs in 32 to 52% of RMSF patients, but hypofibrinogenemia occurs rarely (38, 62, 67, 70). This situation should be contrasted with acute fulminant meningococemia with true disseminated intravascular coagulation in which thrombocytopenia, hypofibrinogenemia, and the pathologic deposition of fibrin in previously uninjured blood vessels are the rule. Hemostasis is an important mechanism for the host; fibrin-platelet thrombi reduce the loss of blood via hemorrhage. The histopathology of RMSF illustrates a constellation of mechanisms, namely, vascular injury that contains predominantly lymphocytes and macrophages rather than the neutrophil-rich vasculitis seen in immune complex disease and few thrombi that generally do not occlude the lumen. Wolbach concluded, in 1919, that "the lesions of the blood vessels are due to the presence of the parasite" (178).

The pathogenic mechanism by which *R. rickettsii* damages the heavily infected endothelial and vascular smooth-muscle cells has long been an enigma (137). A model was developed from the observation that intravenous injection of massive quantities of viable rickettsiae into mice resulted in death 1 to 24 h later (12). The misfortune is that the model was named the mouse toxin phenomenon; subsequently, the prevalent notion has been that rickettsiae have a toxin. In fact, no exotoxin has ever been isolated from rickettsiae, and parabiotic chamber experiments argue strongly against the existence of an exotoxin (148). Rickettsial LPS possesses very low endotoxin activity in the quantities that are found in RMSF (68, 120).

Enzyme activities, particularly those of phospholipase A and protease, have been proposed as rickettsial pathogenic mechanisms (146, 157, 169, 173). Winkler and co-workers have made observations that implicate phospholipase activity directly in the damage to the cell membrane by rickettsiae. Their studies with *R. prowazekii* demonstrated that rickettsiae attach to the erythrocyte membrane, which is then lysed by phospholipase A, resulting in the release of free fatty acids from the erythrocyte (172). When a ratio of 50 or more typhus rickettsiae per host cell are centrifuged onto a monolayer of L cells, immediate cytotoxicity ensues, with an associated release of free fatty acids from the L cell (173). Not only does phospholipase play a role in these experimental models of the rickettsia-cell membrane interaction, but also phospholipase activity is detected in cell cultures over the course of infection with *R. prowazekii* (171). In vitro hemolysis and direct cytotoxicity caused by *R. rickettsii* and phospholipase A activity associated with *R. rickettsii*-infected cells have not been reported. However, *R. rickettsii*-mediated cell injury is reduced by compounds which have been reported to inhibit phospholipase activity or to block attachment of *R. prowazekii* to erythrocytes (146). Clearly, the critical questions related to the role of phospholipase activity in the pathogenesis of RMSF have yet to be answered. Ultrastructural observations support the hypothesis that cell membrane injury is mediated by phospholipase upon rickettsial entry or exit from the host cell (126, 141). Lysed host cell membranes are seen adjacent to *R. rickettsii* in filopodia from which rickettsiae escape, and the presence of dilated rough endoplasmic reticulum in heavily parasitized cells is a common adaptive mechanism of cells to the influx of water. Likewise, reduction in cell injury

by inhibitors of trypsinlike proteases supports the hypothesis that specific proteolytic activity is a physiologic or pathogenic mechanism essential to the expression of cell injury by *R. rickettsii* (157). Other in vitro pathologic phenomena suggest possibly interrelated roles for membrane lipid peroxidation, free radicals, oxygen, and iron in cell injury associated with infection by *R. rickettsii* (124; D. H. Walker, W. T. Firth, and B. C. Hegarty, Abstr. Lab. Invest. 46:86A, 1982). The hypothesis that *R. rickettsii* might damage host cells by parasitism of adenosine triphosphate, amino acids, or other components, leading to a reduction in intracellular pools of a critical metabolite, has not been evaluated adequately. Obligate intracellular parasites offer a serious challenge to the microbiologist ready to study microbial pathogenic mechanisms. Investigation of the highly pathogenic rickettsiae can have an impact on the outcome of severe disease.

Because all strains of *R. rickettsii* that have been isolated from humans in the United States show minimal genetic variability, it may be concluded that, aside from the effects of the quantity of inoculated rickettsiae, the major variables accounting for severity of disease are host factors. Substantially higher fatality/case ratios have been observed for adults than children and for males than females (53). Glucose-6-phosphate dehydrogenase deficiency, a sex-linked genetic condition found in 12% of American black males, predisposes individuals to RMSF of enhanced severity, including higher mortality and, in some patients, a fulminant course leading to death within 5 days or less after disease onset (149, 152). The underlying mechanisms of the propensity to more severe illness are not known. However, differences in host defenses according to age and sex, ability to withstand injury according to age, and rickettsial virulence, possibly enhanced by the products of hemolysis in glucose-6-phosphate dehydrogenase deficiency, may be proposed (156).

The greatest factor in reducing the morbidity and mortality of RMSF is treatment with appropriate antimicrobial agents early in the course of illness (51). In the years 1939 to 1945, which preceded the availability of antirickettsial drugs, the mortality rate was 23%. In recent years, the mortality rate has been as low as 3% (31, 62). Nevertheless, previously healthy persons continue to die of RMSF every year, usually because of misdiagnosis and consequent failure to treat with a tetracycline drug or chloramphenicol in a timely manner (54). Empiric antimicrobial coverage for general suspicion of infection often does not include agents that are effective against rickettsiae. Doxycycline (100 mg every 12 h), tetracycline (50 mg/kg per day in four divided doses), and chloramphenicol (50 mg/kg per day in four divided doses) have antirickettsial activity. Treatment with these drugs too late in the course of severe illness may not avert a fatal outcome.

CLINICAL MANIFESTATIONS

RMSF is a protean systemic illness. Although the disease in some patients who have been treated early may be characterized as mild, most patients suffer moderate or severe illness. Asymptomatic infection with *R. rickettsii* has never been convincingly documented. Rickettsiae infect and damage blood vessels throughout the body. The general pathophysiologic effects of the vascular infection are the consequences of increased vascular permeability and include edema in the tissues surrounding the leaky blood vessels, hypovolemia and hypoproteinemia owing to loss of

protein-rich fluid from the plasma into the tissues, diminished serum oncotic pressure, and reduced perfusion of various organs (52). Multifocal lesions are associated with intense localized rickettsial infection of numerous contiguous endothelial cells (144, 145, 155). The incubation period from the inoculation of rickettsiae into the skin by tick bite until onset of symptoms ranges from 2 to 14 days and averages 7 days. At first, the illness is rather nondescript. During the first 3 days, the most prominent clinical manifestations are fever, malaise, and severe headache often accompanied by muscle aches, anorexia, nausea, vomiting, abdominal pain, and photophobia (62, 67).

A rash usually appears on day 3 of illness, although appearance varies from day 1 in 14% of cases to day 6 or thereafter in 20%. Absence of a rash altogether is reported in 9 to 12% of patients (48, 62, 67, 164). So-called spotless fever occurs in higher proportions of fatal cases, older patients, and blacks. Initially, the rash consists of lesions 1 to 5 mm in diameter where dilation of the small blood vessels imparts a pink color to the skin in and surrounding the foci of rickettsial vascular infection (167). At this stage of illness, pressure applied to the pink spot results in temporary blanching of the rash by removal of blood from the dilated vessels. Later in the course, particularly in severely ill patients, a pinpoint hemorrhage occurs in the center of the pink spot where the damage caused by the intense rickettsial infection is most pronounced. At this later stage, compression of the rash does not blanch its color. These small hemorrhagic spots which give the disease its name are observed in approximately half of the patients with RMSF. The rash is an example of a component of the disease in which the clinical manifestations are due directly to the rickettsial damage to the local tissue.

Among the life-threatening clinical features of RMSF, pulmonary involvement is particularly noteworthy (39, 73, 118, 155). The pulmonary microcirculation is a major target of infection by *R. rickettsii* (145). In the most severely ill patients, rickettsial vascular damage causes potentially lethal leakage of edema fluid into the interstitial tissues and airspaces. Recently, pneumonitis has been reported in 17% of patients with RMSF before they are treated. Severe respiratory failure ensues in 12%. Pulmonary involvement may result in cough, dyspnea, pulmonary edema, and infiltrates in chest radiographs. In a study of 131 patients, 9 of 10 who required mechanical ventilation died (67).

Another critical target is the central nervous system. Involvement of the blood vessels in the brain manifests as rickettsial encephalitis, a grave prognostic indicator (13, 62, 63, 67, 88). Clinically recognized encephalitis occurs in 26 to 28% of patients with RMSF, with signs and symptoms including confusion (28%), stupor or delirium (21 to 26%), ataxia (5 to 18%), coma (9 to 10%), and seizures (8%). Coma occurs much more frequently in fatal cases (86%) than in nonfatal cases (6%). As a result of rickettsial infection of blood vessels of the meninges, brain, and spinal cord, the cerebrospinal fluid contains leukocytes usually in the range of 10 to 100 per μ l in 34 to 38% of patients and increased protein concentration in 30 to 35% of patients (56, 67). The clinical picture may suggest a diagnosis of viral encephalitis, meningitis, or meningococcemia with early meningitis. The clinical laboratory examines cerebrospinal fluid and blood from many patients with RMSF before the correct diagnosis is suspected. It is most apparent that this group of patients with a high risk of a fatal outcome would benefit greatly from a rapid laboratory diagnosis leading to specific antirickettsial therapy.

Patients with severe RMSF are likely to develop acute renal failure, principally because leakage of fluid from the injured blood vessels results in a reduced fluid volume within the blood vessels (154). Consequently, vascular perfusion of the kidneys is diminished, urine output diminishes, and metabolic wastes such as urea and creatinine accumulate in the blood. In the extreme situation of hypotensive shock, renal blood flow is curtailed so extensively that renal tubular cells undergo necrosis (20, 154). Acute renal failure indicates a poor prognosis, complicates fluid and electrolyte management, and may require dialysis.

The presence of gastrointestinal symptoms in RMSF correlates with infection of blood vessels in the stomach, intestines, pancreas, and liver (1, 111). The occurrence of nausea or vomiting (38 to 56%), abdominal pain (30 to 34%), and diarrhea (9 to 20%) early in the course before the onset of rash may lead to a misdiagnosis of gastroenteritis or acute surgical abdomen (37, 62, 65, 67, 87). Patients with RMSF have undergone exploratory abdominal surgery for suspected appendicitis, acute cholecystitis, and perforated diverticulitis (14, 37, 65, 87, 136, 151, 153).

CLINICAL DIAGNOSIS

It should be apparent that the diagnosis of RMSF during the early phase of the illness is by no means easy. There are many pitfalls. The physician who has taken a careful history, performed a complete physical examination, and evaluated the appropriate laboratory data may have no specific clues that the diagnosis is RMSF. Only 3% of patients with RMSF have the classic triad of fever, rash, and history of tick bite during the first 3 days of illness, the time during which the patient usually seeks medical care (62). Gastrointestinal, pulmonary, or central nervous system signs and symptoms may mislead the clinician to an erroneous initial diagnosis of nonspecific viral syndrome, gastroenteritis, acute surgical abdomen, bronchitis, pneumonia, meningoencephalitis, or other misdiagnoses (37, 39, 54, 63, 65, 67, 87, 136, 151, 153). A reliance upon classic textbook manifestations of the disease often delays the diagnosis and appropriate lifesaving treatment. Regarding the previously mentioned triad, fever is present from the onset; rash and history of tick bite are frequently not present. Even for patients with a rash, the physician's insistence on the textbook appearance of the eruption first on the extremities and later on the trunk, a petechial component, and involvement of the palms and soles will result in failure or delay in making the diagnosis. A rash is absent in approximately 10% of cases, may appear first on the trunk, lacks petechiae in half of patients with RMSF, and involves the palms and soles of only 36 to 82% of patients who do have a rash (62, 67). Features sometimes relied on for the clinical diagnosis, petechiae and involvement of the palms and soles, tend to develop rather late in the course, sometimes after the point of irreversible injury. Tick bite is generally painless, often goes unnoticed, and may not be recalled by the patient with central nervous system involvement. A history of tick bite is reported in only 60% of patients (53). On the other hand, in regions where ticks are abundant, a history of tick bite may offer little diagnostic predictive value. In a highly endemic area of North Carolina, Wilfert et al. found that a history of tick bite was obtained from 85% of RMSF patients and also from 54% of matched uninfected controls during the period corresponding to the incubation period (165).

Other epidemiologic variables can be used to support a clinical diagnosis but, if considered as absolute determi-

nants, will lead to misdiagnosis. Most cases can be predicted to occur between mid-April and the end of September and in South Atlantic and West South Central regions. However, cases of RMSF have been documented in the fall and winter and in most states (72, 132). The conclusion must be that there is no established clinicoepidemiologic constellation that can be relied on to help diagnose an acceptable proportion of patients with RMSF. Prior to onset of the rash, the differential diagnosis is too extensive to list but would include such diseases as influenza in its early stages. The appearance of the rash and fever should stimulate the consideration of RMSF as a possible diagnosis. The differential diagnosis includes not only the diseases discussed above as pitfalls, but also gram-negative bacterial sepsis, toxic shock syndrome, measles, rubella, secondary syphilis, enteroviral exanthem, leptospirosis, typhoid fever, disseminated gonococcal infection, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, immune complex vasculitis (e.g., systemic lupus erythematosus), infectious mononucleosis, hypersensitivity reaction to drugs, murine typhus, rickettsialpox, recrudescent typhus, and sylvatic *R. prowazekii* infection that is enzootic in flying squirrels. It would be an understatement to proclaim that the physician could use some assistance from the hospital laboratory in establishing the acute diagnosis of RMSF in a timely manner.

LABORATORY DIAGNOSIS

Given the seriousness of RMSF, the difficulty in making a clinical diagnosis, and the vast number of patients with fever and severe headache whose initial diagnostic considerations should rightfully include RMSF, it is unfortunate that a more effective approach has not been developed and generally established for a timely laboratory diagnosis of RMSF. Commercial interests which demur that 600 to 1,200 patients annually cannot produce a profit fail to comprehend that millions of Americans suffer febrile illnesses in the spring and summer. A laboratory test that determines accurately during the first 3 days of illness exactly which patients have RMSF would be used widely.

The standard of diagnostic methods for infectious diseases is the isolation and identification of the etiologic agent. Bacteriology laboratories go to great effort to recover bacteria that have fastidious growth characteristics or are present in low quantity in the clinical specimen. If identified as normal cutaneous flora or an unusual organism of indeterminant significance, the microorganism represents a substantial investment in effort and supplies, with inconclusive clinical importance. In contrast, isolation and identification of *R. rickettsii*, which is always a significant pathogen, are rarely attempted. Consideration of the explanations for this discrepancy reveals the strength of traditional practices and current fashions for tackling certain problems but not others.

In this era of the acquired immunodeficiency syndrome and recommendations for universal guidelines for handling patient specimens, knowledge of laboratory procedures to prevent infection of the laboratory worker and the environment should make approach to the cultivation of rickettsiae more acceptable. Particularly in the preantibiotic era, rickettsiae earned a deadly reputation for killing scientists who worked with them, including Ricketts himself (106). However, even as organisms requiring class 3 level biohazard containment, rickettsiae should be cultivated in excellent medical and public health laboratories. The differences between an ordinary clinical virology laboratory and one that is safe for isolation of rickettsiae are not great.

TABLE 1. Serologic diagnosis of RMSF

Serologic test	Sensitivity (%)	Specificity (%)	Availability	Titer		References
				Diagnostic criterion	Probable category	
IFA	94–100	100	Many reference laboratories and commercial reagents ^a	Fourfold rise or ≥ 64		61, 66, 69, 91, 104, 105
IHA	91–100	99	Few reference laboratories	Fourfold rise	≥ 128	6, 66, 69, 104, 123, 139
Latex agglutination	71–94	96–99	Commercial reagents ^a	Fourfold rise	≥ 128	59, 60, 66, 69, 104
Complement fixation	0–63	100	Few reference laboratories	Fourfold rise or ≥ 16		69, 91, 104, 121, 139
<i>P. vulgaris</i> OX-19 agglutination	70	78	Commercial reagents		Fourfold rise or ≥ 320	61, 66, 79, 139, 160
<i>P. vulgaris</i> OX-2 agglutination	47	96	Commercial reagents		Fourfold rise or ≥ 320	61, 66, 79, 139

^a Integrated Diagnostics, Inc., Baltimore, Md.

Greater challenges lie in the requirements for impeccable technical expertise. A major problem in biohazard containment is inattention to careful technique or, more forthrightly stated, mental and methodological laxity. *Salmonella typhi* is no longer sent to laboratories as an unknown organism for demonstration of proficiency because of frequent laboratory infections. This problem may be and should be addressed. The second technical problem is similar; only the culture itself is at risk of contamination. Cell culture became easier to perform when antibiotics were developed and added to cell cultures to prevent or suppress bacterial and mycotic contamination. Isolation of rickettsiae in cell culture can be achieved in 4 to 7 days in many cases, but use of antibiotic-free cell culture is mandatory because most antimicrobial agents inhibit rickettsial growth in vitro (41, 68). The reliance on antimicrobial agents in the medium to cover up for minor lapses in technique and contamination with normal flora is virtually universal. Cultivation of rickettsiae depends on meeting the technical challenge of antimicrobial agent-free cell culture. The work should be performed in a biohazard containment safety cabinet in a room under relative negative pressure with an anteroom. The worker must wear mask, gloves, and gown. *R. rickettsii* can be isolated from blood, plasma, and tissues in Vero cells, L cells, primary chicken embryo fibroblasts, and other primary and continuous cell lines (41, 68). The shell vial centrifugation method offers an attractive approach (78). Rickettsiae can also be isolated by inoculation of the yolk sac of antibiotic-free, 5- to 6-day-old embryonated hen eggs (41). Cultivated rickettsiae are identified provisionally by staining with immunofluorescence and the Gimenez method.

Institutions with facilities for handling animals infected with biohazard class 3 agents can isolate *R. rickettsii* from human blood or tissues by intraperitoneal inoculation of adult male guinea pigs (41). Infected animals develop fever of $>40^{\circ}\text{C}$ and rickettsia-induced edema of the scrotum, which sometimes proceeds to hemorrhage and necrosis. Rickettsiae are demonstrable in smears and frozen sections of tunica vaginalis, epididymis, and spleen within days after the onset of fever in the animal. Animals that survive are bled, and convalescent sera are examined for antibodies to *R. rickettsii*. For major medical centers in endemic regions, maintenance of a few adult male guinea pigs during May to September for isolation of *R. rickettsii* offers the opportunity to make the definitive diagnosis of RMSF. Reluctance to work with animals impairs the ability to investigate infectious diseases. The isolation and identification of the etiologic agent of Legionnaires disease was achieved by the inoculation of guinea pigs by a rickettsiologist, Joseph

McDade, after many attempts utilizing bacteriologic media and cell cultures had failed (83). It is important to maintain sensitive methods of recovering fastidious microorganisms such as rickettsiae even if they are quite old-fashioned; it is erroneous to believe that they have been supplanted by other more sensitive methods.

Serologic Diagnosis

In most hospitals the laboratory diagnosis of RMSF is synonymous with the archaic, nonspecific, insensitive Weil-Felix test. Early in this century, the agglutination of certain strains of *Proteus vulgaris* by sera of patients convalescent from typhus fever was recognized (160). This phenomenon depends on antigens shared by *P. vulgaris* OX-19 and OX-2 and *R. prowazekii*, *R. typhi*, *R. rickettsii*, *R. conorii*, *R. sibirica*, and *R. australis*. Between 5 and 12 days after onset of symptoms, antibodies appear that agglutinate *P. vulgaris* OX-19 in 70% of patients and agglutinate *P. vulgaris* OX-2 in 47% (41, 66). In addition to this poor level of sensitivity, another drawback is lack of specificity. Many healthy persons have agglutinating antibodies to *P. vulgaris* OX-19. Among healthy school children, 19.6% had a positive Weil-Felix test at a titer of ≥ 160 (79). Among patients tested for agglutinating antibodies to *P. vulgaris* OX-19, only 35% in a highly endemic region and 3% in New York who had a Weil-Felix titer of ≥ 160 had specific antibodies to antigens of *R. rickettsii* (61, 139). One is forced to conclude that the Weil-Felix test should never be used to diagnose RMSF during the acute phase; both false-positive and false-negative results occur too frequently. Although a fourfold rise in titer detected during convalescence is more specific (70%) and antibodies to *Proteus* antigens decline rapidly in titer over the ensuing months, the level of sensitivity is lower than those of several assays that use specific rickettsial antigens themselves.

Improved specificity and, ultimately, sensitivity were achieved by the use of antigens of *R. rickettsii* itself in assays for antibodies diagnostic of RMSF (Table 1). For a generation, the complement fixation test, using a soluble mixture of ether-extracted protein and LPS antigens of *R. rickettsii*, was the specific serologic method (121). This preparation contains antigens that are shared among *R. rickettsii*, *R. conorii*, *R. sibirica*, *R. akari*, and *R. australis*. Washed rickettsial bodies may be used to detect antibodies to species-specific antigens, but more effort is required to produce them. The complement fixation test is highly reproducible and specific. False-positive results are rare at a serum dilution of 1:16. Unfortunately, the sensitivity of the test is

poor because complement fixation antibodies are detected late in convalescence and in a relatively small proportion of patients (69, 91, 104, 139). Another drawback is that 5 to 10% of sera are anticomplementary; that is, they fix complement even in the absence of rickettsial antigen. The complement fixation test is rarely performed in the United States at present. It has become mainly a subject of historic concern because the major producer of rickettsial antigen for the assay, the CDC, recently ceased supplying the antigen to laboratories in this country. The choice among the other methods for detecting antibodies to rickettsial antigens themselves is determined by availability of reagents and comparison of sensitivities and specificities of the assays.

Agglutination by convalescent serum of microorganisms that are the etiologic agents of an infection was one of the earliest assays for antibodies to antigens of the microorganism itself. Unfortunately, it is virtually impossible to produce sufficient quantities of purified rickettsiae to use diagnostic agglutination tests routinely even with micromethods (45). Cultivation and purification of large concentrations of *R. rickettsii* require particular care to avoid inhalation of infectious aerosols. In addition, these pathogenic organisms destroy the host cells in which they are cultivated before a substantial quantity of rickettsiae have accumulated, and separation of these obligate intracellular bacteria from host cell components is quite inefficient. It is unreasonable to believe that *R. rickettsii* will ever be commercially available for the microagglutination assay. Although the assay is specific, the sensitivity has varied according to the methods of purification of the rickettsiae (91, 104). At a titer of ≥ 32 , the sensitivity of the assay for diagnosis of RMSF was reported as 56%.

In recent years, the principal tests that use antigens of *R. rickettsii* for confirmation of the diagnosis of RMSF are the indirect immunofluorescent antibody assay (IFA), indirect hemagglutination assay (IHA), and latex agglutination. The highest sensitivities are obtained with IFA (94 to 100%) and IHA (91 to 100%) (66, 104, 139). IFA reagents are produced for public health laboratories by the CDC and are also available commercially. The latex agglutination test is an attractive choice because the reagents are available commercially; the assay is simple, requiring no expensive specialized equipment; and the sensitivity of the assay is good. The reagents for IHA are not available commercially.

The IFA is performed on whole rickettsiae, which contain a vast array of protein and carbohydrate antigens. The organisms are affixed to microscopic slides and are reacted with serial dilutions of serum. The presence of antibody attached to the rickettsia is detected by fluorescein-conjugated antibodies to human immunoglobulins (105). Variations in the technique have included detection of antibody of the immunoglobulin M class and demonstration of antibodies by immunoperoxidase instead of immunofluorescence. The availability of an ultraviolet microscope is required for the IFA test. Variations in the endpoint titer may occur because of differences in the quality of the microscope, the quality of the anti-immunoglobulin conjugate, and the skill of the microscopist. The IFA is generally accepted as the best serologic test presently available, the one with which new assays should be compared.

Both IHA and latex agglutination rely on a common source of rickettsial antigen, a protein-carbohydrate complex extracted from *R. rickettsii* by heat and alkaline conditions (32). This antigenic material is coated onto sheep or human type O erythrocytes for IHA and onto latex beads for latex agglutination (6, 59, 60). Serologic reactivity and bind-

ing to the erythrocytes require carbohydrate constituents, and trypsin digestion of the protein constituents does not affect the reaction of the antigen with antibody. Even though IHA demonstrates the earliest, steepest rise in antibody titer of all serologic tests for RMSF, it is seldom diagnostic in the acute stage of illness (69, 139). The median antibody titer during days 4 to 6 of illness, when antirickettsial treatment must be initiated, is 16; only 19% of patients with RMSF had an acute titer of 40, a lower value than the CDC criterion for the single titer indicating a probable diagnosis (≥ 128).

The latex agglutination test is technically simple and rapid and requires no elaborate equipment. Endpoint determination seems to be difficult for some technologists. Antibodies to *R. rickettsii* are detected 7 to 9 days after onset of illness and fall to nondiagnostic titers within 2 months (59, 60). Persistently detectable antibodies by IFA make this test appropriate for study of the prevalence of antibodies. The latex agglutination test is inappropriate for serosurveys and is more diagnostically discriminatory for establishing the diagnosis of a recent infection. It is quite logical that hospital laboratories should replace the insensitive nonspecific Weil-Felix test with the commercially available latex agglutination test.

The most important concept regarding serologic diagnosis is the emphatic insistence that it must be regarded as a retrospective confirmation of the clinical diagnosis. Currently available serologic methods should not be considered as rapid acute diagnostic tests. Very seldom are specific antibodies to *R. rickettsii* detected during the acute stage of illness when empiric treatment must be begun. Most patients with antibodies detected by the Weil-Felix test during the acute stage of illnesses do not turn out to have RMSF upon subsequent testing. Antirickettsial treatment should never be withheld pending a serologic diagnosis. Nevertheless, testing of paired sera by the best test available to demonstrate seroconversion is an important contribution to the patient's record, the physician's data base, and the epidemiologic analyses conducted by public health agencies.

The future prospects for improved serodiagnostic methods for RMSF are quite good in theory. Molecular cloning of recombinant DNA of *R. rickettsii* in expression vectors and development of synthetic peptides corresponding to rickettsial antigenic proteins offer approaches to the development of abundant standardized reagents for detection of antibodies directed against specific epitopes of *R. rickettsii* and antigens shared among rickettsiae of the spotted fever group (8, 85; B. Anderson, Abstr. Seventh Natl. Conf. Am. Soc. Rickettsiol. Rickettsial Dis. 1988, abstr. no. 56, p. 15). Moreover, automated methods with quantitative endpoints on a continuous scale, such as enzyme immunoassays in which these antigens are measured by automated enzyme-linked immunosorbent assay readers, will be feasible (33).

Visualization of Organisms in Tissues

After the development of the Giemsa method for demonstration of *R. rickettsii* by Wolbach (177, 178), for three decades there was no pressure to apply the technique as an acute diagnostic test because there was no effective treatment. Subsequently, the histologic demonstration of rickettsiae by Giemsa stain in infected tissues has become virtually a lost art. The Brown-Hopps method, a tissue Gram stain, detects only a small portion of the organisms that are observed by immunofluorescence. Skin biopsy diagnosis of RMSF by identification of *R. rickettsii* in vascular endothelium and smooth muscle became practical when the immu-

nofluorescent staining method was applied. Coons et al. had visualized *R. rickettsii* by immunofluorescence in 1950 (35). However, not until 1976 were spotted fever rickettsiae identified by immunofluorescence diagnostically in cutaneous biopsies and autopsy tissues (48, 143, 144, 179).

Extensive experience with this acute diagnostic approach led to its use as a routine procedure in some medical centers (68, 139). Best results are obtained by selection of a classic petechial lesion centered in an erythematous maculopapule and removal of this sample of skin with a 3-mm punch under local anesthesia. The sample is mounted in polyethylene glycol; frozen sections are cut perpendicular to the epidermal layer at 4- μ m thickness at 5 to 12 levels extending through the petechia or through two-thirds of the tissue of a nonpetechial lesion. After fixation in absolute acetone for 10 min and air drying, the sections are reacted with an anti-spotted fever group rickettsial antibody conjugated with fluorescein isothiocyanate for 30 min, washed in phosphate-buffered saline for 30 min, and mounted under a cover slip for fluorescence microscopy. A well-characterized conjugate prepared at the CDC has been in standard use for 13 years (58). Undoubtedly, its presently depleted stocks need to be replaced soon by a new stock of polyclonal or monoclonal antibodies for the immunohistologic diagnostic demonstration of *R. rickettsii*. It would be a boon if a well-validated kit were available commercially for the immunohistologic diagnosis of RMSF. RMSF is diagnosed when three or more fluorescent structures compatible with rickettsiae are identified in the blood vessel wall in the dermis. With experience, the test is highly specific (100%) and sufficiently sensitive (70%) to be useful in making clinical decisions. False-negative results increase after 48 h of antirickettsial treatment. Because treatment with antirickettsial drugs for 24 h does not seem to affect the sensitivity of the assay, treatment should not be withheld pending performance of a biopsy. Rickettsiae are cleared from the tissues of experimentally infected guinea pigs treated with tetracycline for 72 h; thus, positive results should not be expected in patients treated for 72 h or longer with an appropriate antirickettsial agent (142). A positive result is diagnostic. A negative result should not preclude empiric antirickettsial treatment based on clinical suspicion of RMSF; however, it should stimulate reconsideration of alternative diagnoses. A method has been developed for the demonstration of *R. rickettsii* in Formalin-fixed, paraffin-embedded tissue. This procedure does not provide as rapid a diagnosis but could be applied to rapid fixation and embedding procedures for same-day or next-day diagnosis. This method often proves useful in establishing the diagnosis after postmortem examination raises the suspicion of undiagnosed RMSF (49, 140).

FUTURE PROSPECTS

The availability of new scientific approaches to the old problems posed by RMSF is encouraging. Detection of specific rickettsial antigens in serum or urine is quite feasible if the necessary scientific effort is expended to accomplish the task (46, 47). Detection of specific rickettsial nucleic acid sequences would probably require examining biopsies of rickettsia-infected lesions in tissue rather than blood. The vast majority of rickettsiae are present within endothelial cells; only a small quantity are circulating in the blood at any given moment of sampling (68). One approach that has been developed but apparently never established in a hospital setting is the detection of diagnostic metabolic products of rickettsiae or pathologic products of the rickettsia-host cell

interaction (84). DNA probes, monoclonal antibodies, a good animal model, and human patients with RMSF are available for the microbiologist who is ready to enter the arena. The study of intracellular bacteria, chlamydiae, rickettsiae, coxiellae, and ehrlichiae, the obligate intracellular pathogens of humans, as well as legionellae, mycobacteria, and other facultative intracellular bacteria requires an infusion of new scientists now. A timely routine method for diagnosing RMSF and other rickettsial diseases in the hospital microbiology laboratory needs to be developed.

ACKNOWLEDGMENTS

I thank Washington Winn, Helen Lucia, Michael McGinnis, Jose Ignacio Herrero-Herrero, Kenneth Gage, Kimberly Weiss, and Joseph McDade for helpful suggestions regarding this manuscript and Rose Dunn for expert secretarial assistance in its preparation.

LITERATURE CITED

1. Adams, J. S., and D. H. Walker. 1981. The liver in Rocky Mountain spotted fever. *Am. J. Clin. Pathol.* **75**:156-161.
2. Anacker, R. L., R. H. List, R. E. Mann, S. F. Hayes, and L. A. Thomas. 1985. Characterization of monoclonal antibodies protecting mice against *Rickettsia rickettsii*. *J. Infect. Dis.* **151**:1052-1060.
3. Anacker, R. L., R. H. List, R. E. Mann, and D. L. Wiedbrauk. 1986. Antigenic heterogeneity in high- and low-virulence strains of *Rickettsia rickettsii* by monoclonal antibodies. *Infect. Immun.* **51**:653-660.
4. Anacker, R. L., R. E. Mann, and C. Gonzales. 1987. Reactivity of monoclonal antibodies to *Rickettsia rickettsii* with spotted fever and typhus group rickettsiae. *J. Clin. Microbiol.* **25**:167-171.
5. Anacker, R. L., G. A. McDonald, R. H. List, and R. E. Mann. 1987. Neutralizing activity of monoclonal antibodies to heat-sensitive and heat-resistant epitopes of *Rickettsia rickettsii* surface proteins. *Infect. Immun.* **55**:825-827.
6. Anacker, R. L., R. N. Philip, L. A. Thomas, and E. A. Casper. 1979. Indirect hemagglutination test for detection of antibody to *Rickettsia rickettsii* in sera from humans and common laboratory animals. *J. Clin. Microbiol.* **10**:677-684.
7. Anacker, R. L., R. N. Philip, J. C. Williams, R. H. List, and R. E. Mann. 1984. Biochemical and immunochemical analysis of *Rickettsia rickettsii* strains of various degrees of virulence. *Infect. Immun.* **44**:559-564.
8. Anderson, B. E., R. L. Regnery, G. M. Carlone, T. Tzianabos, J. E. McDade, Y. F. Zhang, and W. J. Bellini. 1987. Sequence analysis of the 17-kilodalton-antigen gene from *Rickettsia rickettsii*. *J. Bacteriol.* **169**:2385-2390.
9. Austin, F. E., J. Turco, and H. H. Winkler. 1987. *Rickettsia prowazekii* requires host cell serine and glycine for growth. *Infect. Immun.* **55**:240-244.
10. Austin, F. E., and H. H. Winkler. 1988. Relationship of rickettsial physiology and composition to the rickettsia-host cell interaction, p. 29-50. *In* D. H. Walker (ed.), *Biology of rickettsial diseases*, vol. 2. CRC Press, Boca Raton, Fla.
11. Bell, E. J., G. M. Kohls, H. G. Stoenner, and D. B. Lackman. 1963. Nonpathogenic rickettsias related to the spotted fever group isolated from ticks, *Dermacentor variabilis* and *Dermacentor andersoni* from eastern Montana. *J. Immunol.* **90**:770-781.
12. Bell, E. J., and E. G. Pickens. 1953. A toxic substance associated with the rickettsias of the spotted fever group. *J. Immunol.* **70**:461-472.
13. Bell, W. E., and A. D. Lascari. 1970. Rocky Mountain spotted fever, neurological symptoms in the acute phase. *Neurology* **20**:841-847.
14. Benson, M., and D. H. Walker. 1984. Rocky Mountain spotted fever in the differential diagnosis of the acute abdomen. *Contemp. Surg.* **24**:79-83.
15. Bovarnick, M. R. 1956. Phosphorylation accompanying the oxidation of glutamate by the Madrid E strain of typhus

- rickettsiae. *J. Biol. Chem.* **220**:353-361.
16. **Bovarnick, M. R., and J. C. Miller.** 1950. Oxidation and transamination of glutamate by typhus rickettsiae. *J. Biol. Chem.* **184**:661-676.
17. **Bovarnick, M. R., and L. Schneider.** 1960. The incorporation of glycine-1-C¹⁴ by typhus rickettsiae. *J. Biol. Chem.* **235**:1727-1731.
18. **Bovarnick, M. R., L. Schneider, and H. Walter.** 1959. The incorporation of labeled methionine by typhus rickettsiae. *Biochim. Biophys. Acta* **33**:414-422.
19. **Bovarnick, M. R., and J. C. Snyder.** 1949. Respiration of typhus rickettsiae. *J. Exp. Med.* **89**:561-565.
20. **Bradford, W. D., B. P. Croker, and C. C. Tisher.** 1979. Kidney lesions in Rocky Mountain spotted fever. A light-, immunofluorescence-, and electron-microscopic study. *Am. J. Pathol.* **97**:381-390.
21. **Burgdorfer, W.** 1963. Investigation of "transovarial transmission" of *Rickettsia rickettsii* in the wood tick, *Dermacentor andersoni*. *Exp. Parasitol.* **14**:152-159.
22. **Burgdorfer, W.** 1977. Tick-borne diseases in the United States: Rocky Mountain spotted fever and Colorado tick fever. A review. *Acta Trop.* **34**:103-126.
23. **Burgdorfer, W.** 1988. Ecological and epidemiological considerations of Rocky Mountain spotted fever and scrub typhus. p. 33-50. In D. H. Walker (ed.), *Biology of rickettsial diseases*, vol. 1. CRC Press, Boca Raton, Fla.
24. **Burgdorfer, W., and L. P. Brinton.** 1975. Mechanisms of transovarial infection of spotted fever rickettsiae in ticks. *Ann. N.Y. Acad. Sci.* **266**:61-72.
25. **Burgdorfer, W., J. C. Cooney, A. J. Mavros, W. L. Jellison, and C. Masor.** 1980. The role of cottontail rabbits (*Sylvilagus* spp.) in the ecology of *Rickettsia rickettsii* in the United States. *Am. J. Trop. Med. Hyg.* **29**:686-690.
26. **Burgdorfer, W., K. T. Friedhoff, and J. L. Lancaster, Jr.** 1966. Natural history of tick-borne spotted fever in U.S.A. *Bull. W.H.O.* **35**:149-153.
27. **Burgdorfer, W., S. F. Hayes, and A. J. Mavros.** 1981. Non-pathogenic rickettsiae in *Dermacentor andersoni*: a limiting factor for the distribution of *Rickettsia rickettsii*, p. 585-594. In W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
28. **Burgdorfer, W., S. F. Hayes, L. A. Thomas, and J. L. Lancaster, Jr.** 1981. A new spotted fever group rickettsia from the lone star tick, *Amblyomma americanum*, p. 595-602. In W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
29. **Burgdorfer, W., V. F. Newhouse, E. G. Pickens, and D. B. Lackman.** 1962. Ecology of Rocky Mountain spotted fever in western Montana. I. Isolation of *Rickettsia rickettsii* from wild mammals. *Am. J. Hyg.* **76**:293-301.
30. **Burgdorfer, W., D. F. Sexton, R. K. Gerloff, R. L. Anacker, R. N. Philip, and L. A. Thomas.** 1975. *Rhipicephalus sanguineus*: vector of a new spotted fever group rickettsia in the United States. *Infect. Immun.* **12**:205-210.
31. **Centers for Disease Control.** 1988. Rocky Mountain spotted fever—United States, 1987. *Morbidity and Mortality Weekly Report*. **37**:388-391.
32. **Chang, S. M., E. S. Murray, and J. C. Snyder.** 1954. Erythrocyte-sensitizing substances from rickettsiae of the Rocky Mountain spotted fever group. *J. Immunol.* **73**:8-15.
33. **Clements, M. L., J. S. Dumler, P. Fiset, C. L. Wiseman, Jr., M. J. Snyder, and M. M. Levine.** 1983. Serodiagnosis of Rocky Mountain spotted fever: comparison of IgM and IgG enzyme-linked immunosorbent assays and indirect fluorescent antibody test. *J. Infect. Dis.* **148**:876-880.
34. **Clements, M. L., C. L. Wiseman, Jr., T. E. Woodward, P. Fiset, J. S. Dumler, W. McNamee, R. E. Black, J. Rooney, T. P. Hughes, and M. M. Levine.** 1983. Reactogenicity, immunogenicity, and efficacy of a chick embryo cell-derived vaccine for Rocky Mountain spotted fever. *J. Infect. Dis.* **148**:922-930.
35. **Coons, A. H., J. C. Snyder, F. S. Cheever, and E. S. Murray.** 1950. Localization of antigen in tissue cells. IV. Antigens of rickettsiae and mumps virus. *J. Exp. Med.* **91**:31-38.
36. **Cox, H. R.** 1938. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups. *Public Health Rep.* **53**:2241-2247.
37. **Davis, A. E., and W. D. Bradford.** 1982. Abdominal pain resembling acute appendicitis in Rocky Mountain spotted fever. *J. Am. Med. Assoc.* **247**:2811-2812.
38. **Deep, W. D., H. G. Schreiver, and W. Turner.** 1969. Defibrination syndrome in Rocky Mountain spotted fever. *Va. Med. Mon.* **96**:92-95.
39. **Donohue, J. F.** 1980. Lower respiratory tract involvement in Rocky Mountain spotted fever. *Arch. Intern. Med.* **140**:223-227.
40. **DuPont, H. L., R. B. Hornick, A. T. Dawkins, G. G. Heiner, I. B. Fabrikant, C. L. Wiseman, Jr., and T. E. Woodward.** 1973. Rocky Mountain spotted fever: a comparative study of the active immunity induced by inactivated and viable pathogenic *Rickettsia rickettsii*. *J. Infect. Dis.* **128**:340-344.
41. **Elisberg, B. L., and F. M. Bozeman.** 1979. The rickettsiae, p. 1061-1108. In E. H. Lennette and N. J. Schmidt (ed.), *Diagnostic procedures for viral, rickettsial and chlamydial infections*, 5th ed. American Public Health Association, Washington, D.C.
42. **Feng, H. M., C. Kirkman, and D. H. Walker.** 1986. Radioimmunoprecipitation of [³⁵S]methionine-radiolabeled *Rickettsia conorii* and *Rickettsia rickettsii* proteins. *J. Infect. Dis.* **154**:717-721.
43. **Feng, H. M., D. H. Walker, and J. G. Wang.** 1987. Analysis of T-cell-dependent and -independent antigens of *Rickettsia conorii* with monoclonal antibodies. *Infect. Immun.* **55**:7-15.
44. **Feng, W. C., E. S. Murray, W. Burgdorfer, J. M. Spielman, G. Rosenberg, K. Dang, C. Smith, C. Spickert, and J. Waner.** 1980. Spotted fever group rickettsiae in *Dermacentor variabilis* from Cape Cod, Massachusetts. *Am. J. Trop. Med. Hyg.* **29**:691-694.
45. **Fiset, P., R. A. Ormsbee, R. Silberman, M. Peacock, and S. H. Spielman.** 1969. A microagglutination technique for detection and measurement of rickettsial antibodies. *Acta Virol.* **13**:60-66.
46. **Fleck, L.** 1947. Specific antigenic substances in the urine of typhus patients. *Tex. Rep. Biol. Med.* **5**:168-172.
47. **Fleck, L., S. Porat, Z. Evenchik, and M. A. Klingberg.** 1960. The renal excretion of specific microbial substances during the course of infection with murine typhus rickettsiae. *Am. J. Hyg.* **72**:351-361.
48. **Green, W. R., D. H. Walker, and B. G. Cain.** 1978. Fatal viscerotropic Rocky Mountain spotted fever: report of a case diagnosed by immunofluorescence. *Am. J. Med.* **64**:523-528.
49. **Hall, W. C., and L. R. Bagley.** 1978. Identification of *Rickettsia rickettsii* in Formalin-fixed, paraffin-embedded tissues by immunofluorescence. *J. Clin. Microbiol.* **8**:242-245.
50. **Harden, V. A.** 1987. Koch's postulates and the etiology of rickettsial diseases. *J. Hist. Med. Allied Sci.* **42**:277-295.
51. **Harrell, G. T.** 1949. Rocky Mountain spotted fever. *Medicine (Baltimore)* **28**:333-370.
52. **Harrell, G. T., and J. K. Aikawa.** 1949. Pathogenesis of circulatory failure in Rocky Mountain spotted fever. *Arch. Intern. Med.* **83**:331-347.
53. **Hattwick, M. A. W., R. J. O'Brien, and B. F. Hanson.** 1976. Rocky Mountain spotted fever: epidemiology of an increasing problem. *Ann. Intern. Med.* **84**:732-739.
54. **Hattwick, M. A. W., H. Retalliau, R. J. O'Brien, M. Slutzker, R. E. Fontaine, and B. Hanson.** 1978. Fatal Rocky Mountain spotted fever. *J. Am. Med. Assoc.* **240**:1499-1503.
55. **Hayes, S. F., and W. Burgdorfer.** 1982. Reactivation of *Rickettsia rickettsii* in *Dermacentor andersoni* ticks: an ultrastructural analysis. *Infect. Immun.* **37**:779-785.
56. **Haynes, R. E., D. Y. Sanders, and H. G. Cramblett.** 1970. Rocky Mountain spotted fever. Comments on recognition and management based on a study of 63 patients. *Clin. Pediatr.* **17**:685-693.
57. **Hazard, G. W., R. N. Ganz, R. W. Nevin, A. H. Nauss, E. Curtis, W. J. Bell, and E. S. Murray.** 1969. Rocky Mountain spotted fever in the eastern United States. Thirteen cases from

- the Cape Cod area of Massachusetts. *N. Engl. J. Med.* **280**:57-62.
58. Hebert, G. A., T. Tzianabos, W. C. Gamble, and W. A. Chappell. 1980. Development and characterization of high-titered, group-specific fluorescent-antibody reagents for direct identification of rickettsiae in clinical specimens. *J. Clin. Microbiol.* **11**:503-507.
 59. Hechemy, K. E., R. L. Anacker, R. N. Philip, K. T. Kleeman, J. N. MacCormack, S. J. Sasowski, and E. E. Michaelson. 1980. Detection of Rocky Mountain spotted fever antibodies by a latex agglutination test. *J. Clin. Microbiol.* **12**:144-150.
 60. Hechemy, K. E., E. E. Michaelson, R. L. Anacker, M. Zdeb, S. J. Sasowski, K. T. Kleeman, J. M. Joseph, J. Kudlac, L. B. Elliott, J. Rawlings, C. E. Crump, J. D. Folds, H. Dowda, J. H. Barrick, J. R. Hindman, G. E. Killgore, D. Young, and R. H. Altieri. 1983. Evaluation of latex-*Rickettsia rickettsii* test for Rocky Mountain spotted fever in 11 laboratories. *J. Clin. Microbiol.* **18**:938-946.
 61. Hechemy, K. E., R. W. Stevens, S. Sasowski, E. E. Michaelson, E. A. Casper, and R. N. Philip. 1979. Discrepancies in Weil-Felix and microimmunofluorescence test results for Rocky Mountain spotted fever. *J. Clin. Microbiol.* **9**:292-293.
 62. Helmick, C. G., K. W. Bernard, and L. J. D'Angelo. 1984. Rocky Mountain spotted fever: clinical, laboratory, and epidemiological features of 262 cases. *J. Infect. Dis.* **150**:480-488.
 63. Horney, L. F., and D. H. Walker. 1988. Meningoencephalitis as a major manifestation of Rocky Mountain spotted fever. *South. Med. J.* **81**:915-918.
 64. Hughes, L. E., C. M. Clifford, R. Gresbrink, L. Thomas, and J. E. Keirans. 1976. Isolation of a spotted fever group rickettsia from the Pacific Coast tick, *Ixodes pacificus*, in Oregon. *Am. J. Trop. Med. Hyg.* **25**:513-516.
 65. Jimenez, J., W. J. Byrne, J. J. Seibert, and A. R. Euler. 1982. Gastrointestinal symptoms in Rocky Mountain spotted fever. *Clin. Pediatr.* **21**:581-584.
 66. Kaplan, J. E., and L. B. Schonberger. 1986. The sensitivity of various serologic tests in the diagnosis of Rocky Mountain spotted fever. *Am. J. Trop. Med. Hyg.* **35**:840-844.
 67. Kaplowitz, L. G., J. J. Fischer, and P. F. Sparling. 1981. Rocky Mountain spotted fever: a clinical dilemma. *Curr. Clin. Top. Infect. Dis.* **2**:89-108.
 68. Kaplowitz, L. G., J. V. Lange, J. J. Fischer, and D. H. Walker. 1983. Correlation of rickettsial titers, circulating endotoxin, and clinical features in Rocky Mountain spotted fever. *Arch. Intern. Med.* **143**:1149-1151.
 69. Kleeman, K. T., J. L. Hicks, R. L. Anacker, R. N. Philip, E. A. Casper, K. E. Hechemy, C. M. Wilfert, and J. N. MacCormack. 1981. Early detection of antibody to *Rickettsia rickettsii*: a comparison of four serological methods; indirect hemagglutination, indirect fluorescent antibody, latex agglutination, and complement fixation, p. 171-178. *In* W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
 70. Kurnick, J. E., S. H. Malinow, and M. C. Snyderman. 1974. Disseminated intravascular coagulation in Rocky Mountain spotted fever. *South. Med. J.* **67**:623-625.
 71. Lange, J. V., and D. H. Walker. 1984. Production and characterization of monoclonal antibodies to *Rickettsia rickettsii*. *Infect. Immun.* **46**:289-294.
 72. Lange, J. V., D. H. Walker, and T. B. Wester. 1982. Documented Rocky Mountain spotted fever in wintertime. *J. Am. Med. Assoc.* **247**:2403-2404.
 73. Lankford, H. V., and F. L. Glauser. 1980. Cardiopulmonary dynamics in a severe case of Rocky Mountain spotted fever. *Arch. Intern. Med.* **140**:1357-1360.
 74. Li, H., T. R. Jerrells, M. L. Ault, G. L. Spitalny, and D. H. Walker. 1987. Gamma interferon as a crucial host defense against *Rickettsia conorii* in vivo. *Infect. Immun.* **55**:1252-1255.
 75. Li, H., B. Lenz, and D. H. Walker. 1988. Protective monoclonal antibodies recognize heat-labile epitopes on surface proteins of spotted fever group rickettsiae. *Infect. Immun.* **56**:2587-2593.
 76. Lillie, R. D. 1941. I. The pathology of Rocky Mountain spotted fever. *Natl. Inst. Health Bull.* **177**:1-46.
 77. Lou, D., Y. M. Wu, B. Wang, G. D. Liu, J. Z. Li, M. Wang, and Y. F. Han. 1985. A new member of the spotted fever group of rickettsiae—*Rickettsia heilongjiangi*. *Chin. J. Microbiol. Immunol.* **5**:250-253.
 78. Marrero, M., and D. Raoult. 1989. Centrifugation-shell vial technique for rapid detection of spotted fever group rickettsiae in blood culture. *Am. J. Trop. Med. Hyg.* **40**:197-199.
 79. Marx, R. S., C. E. McCall, J. S. Abramson, and J. E. Harlan. 1982. Rocky Mountain spotted fever. Serological evidence of previous subclinical infection in children. *Am. J. Dis. Child.* **136**:16-18.
 80. Maxey, E. E. 1899. Some observations on the so-called spotted fever of Idaho. *Med. Sentinel* **7**:433-438.
 81. McCalla, L. P. 1908. Direct transmission from man to man of the Rocky Mountain spotted (tick) fever. *Med. Sentinel* **16**:87-88.
 82. McDade, J. E., and V. F. Newhouse. 1986. Natural history of *Rickettsia rickettsii*. *Annu. Rev. Microbiol.* **40**:287-309.
 83. McDade, J. E., C. C. Shepard, D. W. Fraser, T. R. Tsai, M. A. Redus, W. R. Dowdle, and Laboratory Investigation Team. 1977. Legionnaires' disease. Isolation of a bacterium and demonstration of its role in other respiratory disease. *N. Engl. J. Med.* **297**:1197-1203.
 84. McDade, J. E., D. E. Wells, J. B. Brooks, and C. C. Alley. 1981. Gas-liquid chromatography of acute-phase serum as a technique for early diagnosis of rickettsial diseases, p. 631-639. *In* W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
 85. McDonald, G. A., R. L. Anacker, and K. Garjian. 1987. Cloned gene of *Rickettsia rickettsii* surface antigen: candidate vaccine for Rocky Mountain spotted fever. *Science* **235**:83-85.
 86. McDonald, G. A., R. L. Anacker, R. E. Mann, and L. J. Milch. 1988. Protection of guinea pigs from experimental Rocky Mountain spotted fever with a cloned antigen of *Rickettsia rickettsii*. *J. Infect. Dis.* **158**:228-231.
 87. Middleton, D. B. 1978. Rocky Mountain spotted fever: gastrointestinal and laboratory manifestations. *South. Med. J.* **71**:629-632.
 88. Miller, J. Q., and T. R. Price. 1972. The nervous system in Rocky Mountain spotted fever. *Neurology* **22**:561-566.
 89. Myers, W. F., and C. L. Wisseman, Jr. 1980. Genetic relatedness among the typhus group of rickettsiae. *Int. J. Syst. Bacteriol.* **30**:143-150.
 90. Myers, W. F., and C. L. Wisseman, Jr. 1981. The taxonomic relationship of *Rickettsia canada* to the typhus and spotted fever groups of the genus *Rickettsia*, p. 313-325. *In* W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
 91. Newhouse, V. F., C. C. Shepard, M. D. Redus, T. Tzianabos, and J. E. McDade. 1979. A comparison of the complement fixation, indirect fluorescent antibody, and microagglutination tests for the serological diagnosis of rickettsial diseases. *Am. J. Trop. Hyg.* **28**:387-395.
 92. Noguchi, H. 1923. Experimental prophylactic inoculation against Rocky Mountain spotted fever. *Northwest Med.* **22**:301-303.
 93. Norment, B. R., and W. Burgdorfer. 1984. Susceptibility and reservoir potential of the dog to spotted fever-group rickettsiae. *Am. J. Vet. Res.* **45**:1706-1710.
 94. Obijeski, J. R., E. L. Palmer, and T. Tzianabos. 1974. Proteins of purified rickettsiae. *Microbios* **11**:61-76.
 95. Ormsbee, R. A. 1985. Rickettsiae as organisms, p. 15-37. *In* J. Kazar (ed.), *Rickettsiae and rickettsial diseases*. Proceedings of the Third International Symposium. Publishing House of the Slovak Academy of Sciences, Bratislava, Czechoslovakia.
 96. Parker, R. R., G. M. Kohls, G. W. Cox, and G. E. Davis. 1939. Observations on an infectious agent from *Amblyomma maculatum*. *Public Health Rep.* **54**:1482-1484.
 97. Parker, R. R., E. G. Pickens, D. B. Lackman, E. J. Bell, and F. B. Thrallkill. 1951. Isolation and characterization of Rocky Mountain spotted fever rickettsiae from the rabbit tick *Haema-*

- physalis leporispalustris* Packard. Public Health Rep. 66:455-463.
98. Parker, R. R., and R. R. Spencer. 1930. Studies on Rocky Mountain spotted fever: results of four years' human vaccination. U.S. Public Health Serv. Hyg. Lab. Bull. 154:72-103.
 99. Pedersen, C. E., and V. C. Walters. 1978. Comparative electrophoresis of spotted fever group rickettsiae. Life Sci. 22: 583-588.
 100. Peter, O., J. C. Williams, and W. Burgdorfer. 1985. *Rickettsia helvetica*, a new spotted fever group rickettsiae: immunochemical analysis of the antigens of 5 spotted fever group rickettsiae, p. 99-105. In J. Kazar (ed.), *Rickettsiae and rickettsial diseases*. Proceedings of the Third International Symposium. Publishing House of the Slovak Academy of Sciences, Bratislava, Czechoslovakia.
 101. Philip, R. N., and E. A. Casper. 1981. Serotypes of spotted fever group rickettsiae isolated from *Dermacentor andersoni* (Stiles) ticks in western Montana. Am. J. Trop. Med. Hyg. 30:230-238.
 102. Philip, R. N., E. A. Casper, R. L. Anacker, J. Cory, S. F. Hayes, W. Burgdorfer, and C. E. Yunker. 1983. *Rickettsia bellii* sp. nov.: a tick-borne rickettsia widely distributed in the United States that is distinct from the spotted fever and typhus biogroups. Int. J. Syst. Bacteriol. 33:94-106.
 103. Philip, R. N., E. A. Casper, W. Burgdorfer, R. R. Gerloff, L. E. Hughes, and E. J. Bell. 1978. Serologic typing of rickettsiae of the spotted fever group by microimmunofluorescence. J. Immunol. 121:1961-1968.
 104. Philip, R. N., E. A. Casper, J. N. MacCormack, D. J. Sexton, L. A. Thomas, R. L. Anacker, W. Burgdorfer, and S. Vick. 1977. A comparison of serologic methods for diagnosis of Rocky Mountain spotted fever. Am. J. Epidemiol. 105:56-67.
 105. Philip, R. N., E. A. Casper, R. A. Ormsbee, M. G. Peacock, and W. Burgdorfer. 1976. Microimmunofluorescence test for the serological study of Rocky Mountain spotted fever and typhus. J. Clin. Microbiol. 3:51-61.
 106. Pike, R. M. 1976. Laboratory-associated infections: summary and analysis of 3921 cases. Health Lab. Sci. 13:106-114.
 107. Popov, V. L., and V. F. Ignatovich. 1976. Electron microscopy of surface structures of *Rickettsia prowazekii* stained with ruthenium red. Acta Virol. 20:424-428.
 108. Price, E. G. 1948. Fighting spotted fever in the Rockies. Naegele Printing Co., Helena, Mont.
 109. Price, W. H. 1953. The epidemiology of Rocky Mountain spotted fever. I. The characterization of strain virulence of *Rickettsia rickettsii*. Am. J. Hyg. 58:248-268.
 110. Ramm, L. E., and H. H. Winkler. 1976. Identification of cholesterol in the receptor site for rickettsiae on sheep erythrocyte membranes. Infect. Immun. 13:120-126.
 111. Randall, M. R., and D. H. Walker. 1984. Gastrointestinal and pancreatic lesions and rickettsial infection in Rocky Mountain spotted fever. Arch. Pathol. Lab. Med. 108:963-967.
 112. Rao, A. K., M. Schapira, M. L. Clements, S. Niewiarowski, A. Budzynski, A. H. Schmaier, P. C. Harpel, W. C. Blackwelder, J.-R. Scherrer, E. Sobel, and R. W. Colman. 1988. A prospective study of platelets and plasma proteolytic systems during the early stages of Rocky Mountain spotted fever. N. Engl. J. Med. 318:1021-1028.
 113. Rees, H. B., Jr., and E. Weiss. 1968. Glutamate catabolism of *Rickettsia rickettsii* and factors affecting retention of metabolic activity. J. Bacteriol. 95:389-396.
 114. Ricketts, H. T. 1906. The study of "Rocky Mountain spotted fever" (tick fever?) by means of animal inoculations. A preliminary communication. J. Am. Med. Assoc. 47:33-36.
 115. Ricketts, H. T., and L. Gomez. 1908. Studies on immunity in Rocky Mountain spotted fever. J. Infect. Dis. 5:221-244.
 116. Robertson, R. G., and C. L. Wisseman, Jr. 1973. Tick-borne rickettsiae of the spotted fever group in West Pakistan. II. Serological classification of isolates from West Pakistan and Thailand: evidence for two new species. Am. J. Epidemiol. 97:55-64.
 117. Rollwagen, F. M., G. A. Dasch, and T. R. Jerrells. 1986. Mechanisms of immunity to rickettsial infection: characterization of a cytotoxic effector cell. J. Immunol. 136:1418-1421.
 118. Sacks, H. S., R. W. Lyons, and B. Lahiri. 1981. Adult respiratory distress syndrome in Rocky Mountain spotted fever. Am. Rev. Respir. Dis. 123:547-549.
 119. Salgo, M. P., E. E. Telzak, B. Currie, D. C. Perlman, N. Litman, M. Levi, G. Nathanson, J. Benach, R. Al-Hafidh, and J. Casey. 1988. A focus of Rocky Mountain spotted fever within New York City. N. Engl. J. Med. 318:1345-1348.
 120. Schramek, S., R. Brezina, and I. N. Tarasevich. 1976. Isolation of a lipopolysaccharide antigen from *Rickettsia* species. Acta Virol. 20:270.
 121. Shepard, C. C., M. A. Redus, T. Tzianabos, and D. T. Warfield. 1976. Recent experience with the complement fixation test in the laboratory diagnosis of rickettsial diseases in the United States. J. Clin. Microbiol. 4:277-283.
 122. Shirai, A., F. M. Bozeman, S. Perri, J. W. Humphries, and H. S. Fuller. 1961. Ecology of Rocky Mountain spotted fever. I. *Rickettsia rickettsii* recovered from a cottontail rabbit from Virginia. Proc. Soc. Exp. Biol. Med. 107:211-214.
 123. Shirai, A., J. W. Dietel, and J. V. Osterman. 1975. Indirect hemagglutination test for human antibody to typhus and spotted fever group rickettsiae. J. Clin. Microbiol. 2:430-437.
 124. Silverman, D. J., and L. S. Santucci. 1988. Potential for free radical-induced lipid peroxidation as a cause of endothelial cell injury in Rocky Mountain spotted fever. Infect. Immun. 56: 3110-3115.
 125. Silverman, D. J., and C. L. Wisseman, Jr. 1978. Comparative ultrastructural study on the cell envelopes of *Rickettsia prowazekii*, *Rickettsia rickettsii*, and *Rickettsia tsutsugamushi*. Infect. Immun. 21:1020-1023.
 126. Silverman, D. J., and C. L. Wisseman, Jr. 1979. In vitro studies of rickettsia-host cell interactions: ultrastructural changes induced by *Rickettsia rickettsii* infection of chicken embryo fibroblasts. Infect. Immun. 26:714-727.
 127. Silverman, D. J., C. L. Wisseman, Jr., A. D. Waddell, and M. Jones. 1978. External layers of *Rickettsia prowazekii* and *Rickettsia rickettsii*: occurrence of a slime layer. Infect. Immun. 22:233-245.
 128. Smith, D. K., and H. H. Winkler. 1977. Characterization of a lysine-specific active transport system in *Rickettsia prowazekii*. J. Bacteriol. 129:1349-1355.
 129. Spencer, R. R., and R. R. Parker. 1923. Rocky Mountain spotted fever: infectivity of fasting and recently fed ticks. Public Health Rep. 38:333-339.
 130. Spencer, R. R., and R. R. Parker. 1924. Experimental studies on tick virus. Public Health Rep. 39:3027-3040.
 131. Spencer, R. R., and R. R. Parker. 1925. Vaccinations of monkeys and man. Public Health Rep. 40:2159-2167.
 132. Taylor, J. P., G. R. Istre, and T. C. McChesney. 1988. The epidemiology of Rocky Mountain spotted fever in Arkansas, Oklahoma, and Texas, 1981 through 1985. Am. J. Epidemiol. 127:1295-1301.
 133. Tyeryar, F. J., Jr., E. Weiss, D. B. Millar, F. M. Bozeman, and R. A. Ormsbee. 1973. DNA base composition of rickettsiae. Science 180:415-417.
 134. Uchida, T., F. Tashiro, T. Funato, and Y. Kitamura. 1986. Isolation of a spotted fever group rickettsia from a patient with febrile exanthematous illness in Shikoku, Japan. Microbiol. Immunol. 30:1323-1326.
 135. Vianna, N. J., and A. R. Hinman. 1971. Rocky Mountain spotted fever on Long Island. Epidemiologic and clinical aspects. Am. J. Med. 51:725-730.
 136. Walker, D. H. 1986. Gastroenterology of Rocky Mountain spotted fever. Pract. Gastroenterol. 10:25-39.
 137. Walker, D. H. 1988. Infectious disease. How do rickettsiae cause disease? An editorial. Yearb. Pathol. Clin. Pathol. 1988:17-21.
 138. Walker, D. H. 1988. Role of the composition of rickettsiae in rickettsial immunity: typhus and spotted fever groups, p. 101-110. In D. H. Walker (ed.), *Biology of rickettsial diseases*, vol. 2. CRC Press, Boca Raton, Fla.
 139. Walker, D. H., M. S. Burday, and J. D. Folds. 1980. Laboratory diagnosis of Rocky Mountain spotted fever. South. Med.

- J. 73:1443-1447.
140. Walker, D. H., and B. G. Cain. 1978. A method for specific diagnosis of Rocky Mountain spotted fever on fixed, paraffin-embedded tissue by immunofluorescence. *J. Infect. Dis.* 137: 206-209.
141. Walker, D. H., and B. G. Cain. 1980. The rickettsial plaque. Evidence for direct cytopathic effect of *Rickettsia rickettsii*. *Lab. Invest.* 43:388-396.
142. Walker, D. H., B. G. Cain, J. V. Lange, and A. Harrison. 1981. Pathologic and rickettsial events in tetracycline-treated experimental Rocky Mountain spotted fever, p. 347-362. In W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
143. Walker, D. H., B. G. Cain, and P. M. Olmstead. 1978. Laboratory diagnosis of Rocky Mountain spotted fever by immunofluorescent demonstration of *Rickettsia rickettsii* in cutaneous lesions. *Am. J. Clin. Pathol.* 69:619-623.
144. Walker, D. H., B. G. Cain, and P. M. Olmstead. 1978. Specific diagnosis of Rocky Mountain spotted fever by immunofluorescent demonstration of *Rickettsia rickettsii* in biopsy of skin. *Public Health Lab.* 36:96-100.
145. Walker, D. H., C. G. Crawford, and B. G. Cain. 1980. Rickettsial infection of the pulmonary microcirculation: the basis of interstitial pneumonitis of Rocky Mountain spotted fever. *Hum. Pathol.* 11:263-272.
146. Walker, D. H., W. T. Firth, J. F. Ballard, and B. C. Hegarty. 1983. Role of the phospholipase-associated penetration mechanism in cell injury by *Rickettsia rickettsii*. *Infect. Immun.* 40:840-842.
147. Walker, D. H., W. T. Firth, and C. J. S. Edgell. 1982. Human endothelial cell culture plaques induced by *Rickettsia rickettsii*. *Infect. Immun.* 37:301-306.
148. Walker, D. H., W. T. Firth, and B. C. Hegarty. 1984. Injury restricted to cells infected with spotted fever group rickettsiae in parabiologic chambers. *Acta Trop.* 41:307-312.
149. Walker, D. H., H. L. Hawkins, and P. Hudson. 1983. Fulminant Rocky Mountain spotted fever. Its pathologic characteristics associated with glucose-6-phosphate dehydrogenase deficiency. *Arch. Pathol. Lab. Med.* 107:121-125.
150. Walker, D. H., and F. W. Henderson. 1978. Effect of immunosuppression on *Rickettsia rickettsii* infection in guinea pigs. *Infect. Immun.* 20:221-227.
151. Walker, D. H., F. W. Henderson, and G. M. Hutchins. 1986. Rocky Mountain spotted fever: mimicry of appendicitis or acute surgical abdomen? *Am. J. Dis. Child.* 140:742-744.
152. Walker, D. H., H. N. Kirkman, and P. H. Wittenberg. 1981. Genetic states possibly associated with enhanced severity of Rocky Mountain spotted fever, p. 621-630. In W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
153. Walker, D. H., H. R. Lesesne, V. A. Varma, and W. C. Thacker. 1985. Rocky Mountain spotted fever mimicking acute cholecystitis. *Arch. Intern. Med.* 145:2194-2196.
154. Walker, D. H., and W. D. Mattern. 1979. Acute renal failure in Rocky Mountain spotted fever. *Arch. Intern. Med.* 139:443-448.
155. Walker, D. H., and W. D. Mattern. 1980. Rickettsial vasculitis. *Am. Heart J.* 100:896-906.
156. Walker, D. H., D. L. Radisch, and H. N. Kirkman. 1983. Haemolysis with rickettsiosis and glucose-6-phosphate dehydrogenase deficiency. *Lancet* ii:217.
157. Walker, D. H., R. R. Tidwell, T. M. Rector, and J. D. Geratz. 1984. The effect of synthetic protease inhibitors of the amidine type on cell injury by *Rickettsia rickettsii*. *Antimicrob. Agents Chemother.* 25:582-585.
158. Walker, T. S. 1984. Rickettsial interactions with human endothelial cells in vitro: adherence and entry. *Infect. Immun.* 44:205-210.
159. Walker, T. S., and H. H. Winkler. 1978. Penetration of cultured mouse fibroblasts (L cells) by *Rickettsia prowazekii*. *Infect. Immun.* 22:200-208.
160. Weil, E., and A. Felix. 1916. Zur serologischen Diagnose des Fleckfiebers. *Wien. Klin. Wochenschr.* 29:33-35.
161. Weiss, E. 1988. History of rickettsiology, p. 33-50. In D. H. Walker (ed.), *Biology of rickettsial diseases*, vol. 1. CRC Press, Boca Raton, Fla.
162. Weiss, E., and J. W. Moulder. 1984. The rickettsias and chlamydias, p. 687-739. In N. R. Kreig and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
163. Weiss, E., H. B. Rees, Jr., and J. R. Hayes. 1967. Metabolic activity of purified suspensions of *Rickettsia rickettsii*. *Nature (London)* 213:1020-1022.
164. Westerman, E. L. 1982. Rocky Mountain spotless fever. A dilemma for the clinician. *Arch. Intern. Med.* 142:1106-1107.
165. Wilfert, C. M., J. N. MacCormack, D. Kleeman, R. N. Philip, E. Austen, V. Dickinson, and L. Turner. 1984. Epidemiology of Rocky Mountain spotted fever as determined by active surveillance. *J. Infect. Dis.* 150:469-479.
166. Williams, J. C., D. H. Walker, M. G. Peacock, and S. T. Stewart. 1986. Humoral immune response to Rocky Mountain spotted fever in experimentally infected guinea pigs: immunoprecipitation of lactoperoxidase ¹²⁵I-labeled proteins and detection of soluble antigens of *Rickettsia rickettsii*. *Infect. Immun.* 52:120-127.
167. Wilson, L. B., and W. M. Chowning. 1904. Studies in *Pyroplasmosis hominis* ("spotted fever" or "tick fever" of the Rocky Mountains). *J. Infect. Dis.* 1:31-57.
168. Winkler, H. H. 1976. Rickettsial permeability and ADP-ATP transport system. *J. Biol. Chem.* 251:389-396.
169. Winkler, H. H. 1985. Rickettsial phospholipase A activity, p. 185-190. In J. Kazar (ed.), *Rickettsiae and rickettsial diseases*. Proceedings of the Third International Symposium. Publishing House of the Slovak Academy of Sciences, Bratislava, Czechoslovakia.
170. Winkler, H. H., and R. M. Daugherty. 1984. Proline transport and metabolism in *Rickettsia prowazekii*. *J. Bacteriol.* 158: 460-463.
171. Winkler, H. H., and R. M. Daugherty. 1989. Phospholipase A activity associated with the growth of *Rickettsia prowazekii* in L929 cells. *Infect. Immun.* 57:36-40.
172. Winkler, H. H., and E. T. Miller. 1980. Phospholipase A activity in the hemolysis of sheep and human erythrocytes by *Rickettsia prowazekii*. *Infect. Immun.* 29:316-321.
173. Winkler, H. H., and E. T. Miller. 1981. Immediate cytotoxicity and phospholipase A: the role of phospholipase A in the interaction of *R. prowazekii* and L-cells, p. 327-333. In W. Burgdorfer and R. L. Anacker (ed.), *Rickettsiae and rickettsial diseases*. Academic Press, Inc., New York.
174. Wisseman, C. L., Jr., E. A. Edlinger, A. D. Waddell, and M. R. Jones. 1976. Infection cycle of *Rickettsia rickettsii* in chicken embryo and L-929 cells in culture. *Infect. Immun.* 14:1052-1064.
175. Wisseman, C. L., Jr., and A. D. Waddell. 1975. In vitro studies on rickettsia-host cell interactions: intracellular growth cycle of virulent and attenuated *Rickettsia prowazekii* in chicken embryo cells in slide chamber cultures. *Infect. Immun.* 11: 1391-1401.
176. Wisseman, C. L., Jr., and A. D. Waddell. 1983. Interferonlike factors from antigen- and mitogen-stimulated human leukocytes with antirickettsial and cytolytic actions on *Rickettsia prowazekii* infected human endothelial cells and fibroblasts. *J. Exp. Med.* 158:1780-1793.
177. Wolbach, S. B. 1916. The etiology of Rocky Mountain spotted fever. A preliminary report. *J. Med. Res.* 34:121-128.
178. Wolbach, S. B. 1919. Studies on Rocky Mountain spotted fever. *J. Med. Res.* 41:2-197.
179. Woodward, T. E., C. E. Pedersen, C. N. Oster, L. R. Bagley, J. Romberger, and M. J. Snyder. 1976. Prompt confirmation of Rocky Mountain spotted fever: identification of rickettsiae in skin tissues. *J. Infect. Dis.* 134:297-301.
180. Yamada, T., P. Harber, G. W. Pettit, D. A. Wing, and C. N. Oster. 1978. Activation of the kallikrein-kinin system in Rocky Mountain spotted fever. *Ann. Intern. Med.* 88:764-768.