Koch's Postulates and the Etiology of Rickettsial Diseases

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IN his famous 1882 paper on the etiology of tuberculosis, Robert Koch described the procedures that were necessary and sufficient to demonstrate bacterial causation for tuberculosis, and by extension, for other bacterial diseases as well. He stated: "It was necessary to isolate the bacilli from the body, to grow them in pure culture until they were freed from any disease-product of the animal organism which might adhere to them; and, by administering the isolated bacilli to animals, to reproduce the same morbid condition which, as known, is obtained by inoculation with spontaneously developed tuberculous material."[1] These criteria, which became known as "Koch's postulates," were predicted on the assumption that microbial pathogens were living cells with predictable behaviors. The second imperative—to obtain a pure culture of the organism—was of key importance, and a "pure culture" was generally accepted to mean a colony of organisms grown on lifeless media, especially on the solid gelatin media developed by Koch himself. Even at the time Koch stated the postulates, the "viruses," or infectious agents, of many diseases—especially those that were invisible under the microscope and passed through filters—could not be cultured

1. Robert Koch, "Die Aetiologie der Tuberculose," *Berl. klin. Wchnschr.* 19: 221–230, 1882, trans. Dr. and Mrs. Max Pinner, idem, *The Aetiology of Tuberculosis*, New York, National Tuberculosis Association, 1922, quotation from p. 31. Lester S. King, in "Dr. Koch's postulates," *J. Hist. Med.*, 7: 350–361, 1952, pointed out that even for bacterial diseases, the postulates had to be understood as a method of elucidating a known disease process, not as a means to define disease.

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on lifeless media. This problem was generally viewed as the result of imperfect technique, and scientists who argued that the filterable viruses were a different type of pathogen were in the minority.[2] Koch died in 1910, just as other investigators were discovering additional minute, but visible, organisms, which like the filterable viruses, stubbornly resisted cultivation on artificial media. These were the suspected pathogens of Rocky Mountain spotted fever and typhus, known today as members of the rickettsial disease group. This paper examines the contribution of rickettsial disease research to the expansion of the concept of "pathogen" and to the impact of this change on the definition of "pure culture" in Koch's second postulate.

The rickettsial diseases are caused by exceptionally small bacteria that metabolize and multiply only inside living cells. They are primarily parasites of arthropod hosts such as ticks, mites, and insects. Mammals are infected through the bite of the host arthropod or by contact with its infectious feces. In nature, most of the rickettsiae are transmitted from generation to generation of host arthropod through the eggs of the female. Rocky Mountain spotted fever rickettsiae inhabit ticks, endemic typhus rickettsiae live in fleas, and tsutsugamushi—better known as scrub typhus—rickettsiae reside in mites. Humans act as the natural reservoir of epidemic typhus, which is spread from host to host by the body louse.

Although epidemic typhus had for centuries ravaged armies, jails, and other places where people were crowded together in unsanitary surroundings, Rocky Mountain spotted fever was the first rickettsial disease to yield a portion of its etiological secret to the scrutiny of bacteriological methods. This mysterious disease, which, like typhus, caused a high fever, headache, and widespread rash, was often encountered by late nineteenth-century settlers in the northwestern United States. In most places its mortality ranged from 5 to 10%, but in the Bitterroot Valley of Montana it killed approximately 70% of its victims. Spotted fever cases in the Bitterroot usually numbered fewer than a dozen a year, but by 1900 an expanding population experiencing economic growth demanded that government authorities take action to eliminate the scourge. The newly created Montana State Board of Health responded to this problem between 1902 and 1905 by engaging the services of two Minnesota pathologists and appealing for help to the United States Public

2. Sally Smith Hughes, *The Virus: A History of the Concept*, New York, Science History Publications, 1977, pp. 29–41, 61–73.

Health Service. These early investigators disagreed, however, over the nature of the etiological agent and its means of transmission.[3]

In 1906 University of Chicago researcher Howard Taylor Ricketts launched a program of study that for the first time illuminated the situation.[4] That year he and Walter W. King of the Public Health Service independently demonstrated that spotted fever was indeed transmitted by a species of tick, later classified as *Dermacentor andersoni*. Ricketts continued the work, and by 1907 he was consistently finding in the blood of victims "small spherical, ovoid and diplococcoid forms" that seemed to be bacteria.[5] Using Giemsa stain, he saw "two somewhat lanceolate chromatin-staining bodies, separated by a slight amount of eosin-staining substance."[6] He hesitated to publish his findings at this point for two reasons. No other known bacterial disease was transmitted biologically through the bite of a tick, and he could not culture the elusive bacterium despite his best efforts.

After another year of research, Ricketts ventured a conservative description of the organism in the *Journal of the American Medical Association*, stopping short of claiming specific etiology. For the moment, he rested his case on the observation that the serum of guinea pigs recovered from a bout with spotted fever produced agglutination at high dilution with the organism in infected tick eggs. "In so far as I know," Ricketts wrote, "it would be an unheard-of circumstance to obtain such strong agglutination with an immune serum, in the presence of negative controls, unless there were a specific relationship between the organism and the disease."[7] To his friends, in private, Ricketts was less cautious. "Just a

3. On this early work see Victoria A. Harden, "Rocky Mountain spotted fever research and the development of the insect vector theory, 1900–1930," *Bull. Hist. Med.*, *59*: 449–466, 1985; Esther Gaskins Price, *Fighting Spotted Fever in the Rockies*, Helena, Montana, Naegele Printing, 1948, pp. 12–38.

4. For biographical information on Ricketts see the *Dictionary of Scientific Biography*, s.v., "Howard Taylor Ricketts," by Pierce Mullen; William K. Beatty and Virginia L. Beatty, "Howard Taylor Ricketts—imaginative investigator," *Proc. Inst. Med. Chicago*, *34*: 46–48, 1981; Ludvig Hektoen's memorial address on Ricketts in Howard T. Ricketts, *Contributions to Medical Science by Howard Taylor Ricketts, 1870–1910*, Chicago, University of Chicago Press, 1911, pp. 3–7; the *Dictionary of American Biography*, s.v. "Howard Taylor Ricketts" by H. Gideon Wells; Paul F. Clark, *Pioneer Microbiologists of America*, Madison, University of Wisconsin Press, 1961, pp. 285–291; obituary in *JAMA*, *54*: 1640, 1910. No book-length biography of Ricketts has yet been written.

5. Howard Taylor Ricketts to Ludvig Hektoen, 4 June 1907, folder 10, box 8, Howard Taylor Ricketts Papers, Department of Special Collections, Joseph Regenstein Library, University of Chicago, Chicago, Illinois. Hereinafter cited as Ricketts Papers.

6. Howard Taylor Ricketts, "A micro-organism which apparently has a specific relationship to Rocky Mountain spotted fever: a preliminary report," *JAMA*, *52*: 379–380, 1909.

7. Ibid.

note to tell you that I have found the microorganism of spotted fever," he wrote to Thomas D. Tuttle, secretary of the Montana State Board of Health, just before this article was published.[8] Two months later, Ricketts reminded the jubilant Tuttle that failure to cultivate the microbe, "and thus meet one of Koch's great laws," made it necessary "to bring all kind of indirect evidence to bear showing that we have the real thing."[9]

Unfortunately, Ricketts's work in 1909 was halted because of fiscal problems in the Montana state legislature. Having appropriated more money than it would receive in tax revenues, the legislature was forced by the State Board of Examiners to rescind all funding for special projects such as spotted fever work until additional revenues were collected. Since Ricketts had no assurance that the legislature would reinstate the money, he accepted an offer from the Mexican government to investigate the etiology and means of transmission of tabardillo, the Mexican typhus fever named for the rash that resembled a red cloak on its victims.[10]

By the time Ricketts arrived in Mexico in December 1909, he undoubtedly knew that he was too late to achieve priority in establishing the mode of transmission of typhus. Earlier in the year French researcher Charles Nicolle, working in the north African city of Tunis, had transmitted the disease from monkey to monkey by means of the body louse.[11] In addition, Public Health Service scientists John F. Anderson and Joseph Goldberger, who worked in Mexico until Goldberger contracted the disease, had confirmed Nicolle's findings and succeeded in directly innoculating the typhus pathogen into monkeys.[12] Gamely proceeding with his work even though he had lost the chance to be first, Ricketts and his assistant Russell M. Wilder also confirmed the earlier findings.[13]

8. Howard Taylor Ricketts to Thomas D. Tuttle, 25 January 1909, folder 1, "Rocky Mountain Spotted Fever, 1908–1911," box 1, "General Correspondence," Montana State Board of Health Records, Record Group 28, Montana State Archives, Helena, Montana. Hereinafter cited as RG 28, Montana State Archives.

9. Howard Taylor Ricketts to Thomas D. Tuttle, 17 March 1909, folder 1, "Rocky Mountain Spotted Fever, 1908–1911," box 1, "General Correspondence," RG 28, Montana State Archives.

10. Information about Montana's financial problems is in folder 1, "Rocky Mountain Spotted Fever, 1908–1911," box 1, "General Correspondence," RG 28, Montana State Archives; Minutes of the State Board of Health, 1 April 1909, Montana State Archives; and in folder 12, box 8, Ricketts Papers. Correspondence about Ricketts's decision to go to Mexico City is in folder 15, box 4 and in folder 12, box 8, Ricketts Papers.

11. Charles Nicolle, C. Comte, and E. Conseil, "Transmission expérimentale du typhus exanthématique par le pou du corps," *Compt. rend. Acad. Sci., 149*: 486–489, 1909.

12. John F. Anderson and Joseph Goldberger, "On the relation of Rocky Mountain spotted fever to the typhus fever of Mexico: a preliminary note," *Public Health Rep., 24*: 1861–1862, 1909; idem, "A note on the etiology of 'tabardillo,' the typhus fever of Mexico," ibid., pp. 1941–1942.

13. The entire series of Ricketts and Wilder's papers on typhus is in Ricketts, (n. 4) *Contributions to Medical Science*, pp. 451–500.

More importantly, Ricketts described an organism similar to the spotted fever germ that was consistently found in the blood of tabardillo patients, in the lice that fed on these patients and in the feces of the infected lice. Using Giemsa stain, he "invariably" found a short bacillus having roughly the same morphology of bubonic plague and Rocky Mountain spotted fever organisms. In addition, he saw "certain other bodies, the identity of which is not so clear," which might represent "degeneration or involution forms" of the organism.[14] Like the spotted fever agent, the presumed bacillus was exceptionally small.

Ricketts fared no better in attempts to culture the bacillus found in typhus blood than he had in spotted fever research. Because of this, he concluded that he lacked grounds sufficient for claiming an etiologic role for the bacillus. Yet from his experience with both spotted fever and typhus, he questioned whether cultivation on artificial media was the only means to satisfy Koch's second postulate. After reviewing his own unsuccessful attempts and those of others, Ricketts concluded simply "that the organism we described is not susceptible to cultivation under ordinary conditions."[15] Tragically, Ricketts never had an opportunity to pursue his reasoning through further experiments. He contracted typhus during his research and died on 3 May 1910.[16]

For the next five years, little progress was made in laboratory studies on typhus or spotted fever. In the Bitterroot Valley, efforts to control spotted fever focused on controlling the tick vector.[17] Lunsford D. Fricks, who implemented this program for the Public Health Service, continued bacteriological studies but had no more luck than his predecessors in cultivating any organism. In early 1916, however, scientists at two other research centers began to study spotted fever. In January, Harvard University Medical School pathologist Simeon Burt Wolbach contacted Fricks to obtain strains of spotted fever in guinea pigs for investigation.[18]

14. Howard Taylor Ricketts and Russell M. Wilder, "The etiology of the typhus fever (tabardillo) of Mexico City: a further preliminary report," *JAMA, 54*: 1373–1375, 1910.

15. Ibid.

16. References to Ricketts's illness and death are in folder 1, "Rocky Mountain Spotted Fever, 1908–1911," box 1, "General Correspondence," RG 28, Montana State Archives; folder 12 box 8, Ricketts Papers.

17. An account of this work stressing the contribution of the Montana State Board of Entomology is in Price, (n. 3) *Fighting Spotted Fever*, pp. 74–141.

18. For biographical information on Wolbach, see Charles A. Janeway, "S. Burt Wolbach, 1880–1954," *Trans. Assoc. Am. Physicians, 67*: 30–35, 1954; Shields Warren, "Simeon Burt Wolbach, 3rd July 1880–19th March 1954," *J. Pathol. Bacteriol., 68*: 656–657, 1954; Sidney Farber and Charlotte L. Maddock, "S. Burt Wolbach, M. D., 1880–1954," *Arch. Pathol., 59*: 624–630, 1955; "S. Burt Wolbach," in Esmond R. Long, *History of the American Society for Experimental Pathology*, Bethesda, Maryland, American Society for Experimental Pathology, 1972, pp. 89–90; "Dr. S. B. Wolbach, Pathologist, Dies," *New York Times*, 20 March 1954, p. 15.

About the same time, bacteriologist Hideyo Noguchi at the Rockefeller Institute for Medical Research in New York likewise decided that the spotted fever problem might be a productive line of research.[19]

Fricks, who had studied the disease since 1913, was prodded into publication of his findings by this competition. In early 1916, at medical meetings in Missoula and Salt Lake City, he announced that he had consistently found "extra corpuscular granules" in the blood of human and animal victims of spotted fever. These, he stated, occurred singly and in pairs, and when stained by the Giemsa method, appeared bright red and were highly refractile. He also found similar bodies "within or in close proximity to" the red blood cells. Those inside the red cell, he said, were "round or slightly elongated red chromatin bodies partially surrounded by or in close approximation to a somewhat larger deep-blue staining body." All of the chromatin bodies, he stated, were one micron or less in diameter. Fricks concluded that the "morphological and tinctorial characteristics" of the bodies he described implied that they were of a protozoan nature. Several protozoa were known to be impossible to cultivate, hence Frick's conclusion adhered to observed behavior.[20]

Wolbach, although just beginning his studies, had little regard for Frick's presumed organism. Corresponding with Montana State Board of Health secretary William F. Cogswell, Wolbach confided: "I am on an entirely different track and have great hopes of contributing something of importance." It would take time, he continued, to confirm his hypotheses, because he was using the "peculiarly difficult technique" of teasing apart tick tissues rather than crushing them.[21] Initially, Wolbach had planned to supplement these laboratory studies of tick and guinea pig tissues by traveling to Montana to study human cases of the disease. Unfortunately, he suffered an attack of appendicitis with complications that precluded any travel.[22]

19. For biographical information on Noguchi see Isabel R. Plesset, *Noguchi and His Patrons*, Rutherford, New Jersey, Fairleigh Dickinson University Press, 1980; Gustav Eckstein, *Noguchi*, New York, Harper, 1931; Paul Franklin Clark, "Hideyo Noguchi, 1876–1928," *Bull. Hist. Med.*, *33*: 18–19, 1959. My discussion of Noguchi's early spotted fever work follows Plesset, pp. 166–173. For an examination of the interactions between Japanese and Western investigators, see Ilza Veith, "On the mutual indebtedness of Japanese and Western Medicine," *Bull. Hist. Med.*, *52*: 383–409, 1978.

20. Lunsford D. Fricks, "Rocky Mountain spotted fever: a report of laboratory investigations of the virus," *Pub. Health Rep.*, *31*: 516–521, 1916.

21. S. B. Wolbach to W. F. Cogswell, 21 February 1916, folder 2, "Rocky Mountain Spotted Fever, 1912–1919," box 1, "General Correspondence," RG 28, Montana State Archives.

22. S. B. Wolbach to R. A. Cooley, 21 February, 21 April 1916; Cooley to Wolbach, telegram, 1 March and letter, 8 June 1916, volume "Professors at Various Universities"; W. F. Cogswell to

Despite this setback, Wolbach determined to publish preliminary findings based solely on studies of tick and guinea pig tissues rather than risk losing priority to Noguchi.[23] His research had revealed, Wolbach wrote to Robert A. Cooley, secretary of the Montana State Board of Entomology, that spotted fever affected "primarily the peripheral blood vessels" and that the rash and necrosis were "secondary to the vascular lesions." These findings were "entirely consistent and confirmatory of clinical descriptions of the disease," he continued, and he expressed surprise that no one had previously paid attention to the tissues, which he regarded as "essential."[24]

In mid-1916, Wolbach published two papers incorporating these preliminary findings in the *Journal of Medical Research*. In the first, he described a Gram negative organism from 0.2 to 0.5 microns wide that occasionally occurred "in enormous numbers" and was concentrated in the "smooth muscle cells of affected arteries and veins." With Giemsa's stain, the organisms stained "bluish," he stated, this being "in marked contrast to most bacteria, which take an intense reddish purple stain." Since this reddish purple coloration—usually achieved by using the Romanowsky stains—was regarded as the chromatin staining reaction, Wolbach noted that he was "somewhat at a loss to understand the description 'chromatin staining' by Ricketts as applied to this organism."[25] This initial paper was followed a few months later by a second preliminary report on the organism in ticks. Although he had observed the organism throughout tick tissues, Wolbach concluded that there was no cellular reaction in the ticks to the presence of the parasites, "even when present in enormous numbers."[26] This was indicative that the organism had evolved a symbiotic relationship with its tick host over the centuries.

S. B. Wolbach, 29 April 1916, volume "W. F. Cogswell, A. H. McCray, T. D. Tuttle," in bound volumes of Robert A. Cooley's correspondence during his tenure as secretary of the Montana State Board of Entomology, 1913–1931, 17 vols. This collection is temporarily located in the office of the historian, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, but in September 1987 it will be transferred to the Montana State Archives, Helena, Montana. Hereinafter cited as Cooley Correspondence.

23. "I hastened into print," Wolbach confided in a letter to Fricks, "because of Noguchi's competition." See S. B. Wolbach to L. D. Fricks, 21 April 1916, file "S. F. History (Correspondence with Wolbach—1916)," historical files at the Rocky Mountain Laboratory, Hamilton, Montana. Hereinafter cited as RML permanent historical files.

24. S. B. Wolbach to R. A. Cooley, 14 November 1916, volume "Professors at Various Universities," Cooley Correspondence.

25. S. Burt Wolbach, "The etiology of Rocky Mountain spotted fever (a preliminary report)," *J. Med. Res.*, *34*: 121–125, 1916.

26. S. Burt Wolbach, "The etiology of Rocky Mountain spotted fever: occurrence of the parasite in the tick (second preliminary report)," *J. Med. Res.*, *35*: 147–150, 1916.

Ironically, although it was Hideyo Noguchi's perceived competition that stimulated the publication of Frick's and Wolbach's papers, Noguchi did not make much progress during 1916 on spotted fever. His attention had turned instead to studies of the spirochete that caused Weil's disease, an organism which he identified as a new genus, *Leptospira*.[27] Having read the papers published by both Fricks and Wolbach, however, Noguchi was inclined to support Fricks's protozoan theory. Upon receiving a slide from Fricks of his organism, Noguchi replied that he, too, had "seen similar bodies several times" in his own work.[28] As a specialist in spirochetes, some of which were known to be arthropod-borne and whose classification as bacteria, protozoa, or something in-between then remained unsettled, it is not surprising that Noguchi was receptive to the possibility of a spotted fever organism with protozoan characteristics.

It was Wolbach's pathological study of tissues rather than bacteriological study of blood that would make the most conclusive case for the unique etiology of spotted fever. As he continued his examination of guinea pig lesions and tick tissues, Wolbach encountered an entirely unanticipated phenomenon that altered his perception of the nature of the disease organism. By December 1916, he was certain that he had seen the organism multiplying "in the nuclei of the Malpighian tubules of ticks. This is the first instance known," he wrote to Robert A. Cooley, "of a parasite multiplying inside of nuclei. As you see," he concluded, "I am getting away from the idea that the organism is a bacterium."[29]

In the spring of 1917, Wolbach was finally able to come to Montana for several weeks, where he conducted autopsies on two spotted fever victims. To his surprise, the lesions of the disease in humans had an "exact similarity" to those in animals. Commenting on this "remarkable feature," of spotted fever, Wolbach asserted: "There is probably no other disease of man which is so accurately duplicated in animals."[30] Furthermore, Wolbach had decided that the spotted fever organism was indeed unique. "My opinion regarding the organism," he wrote Cooley, "is that it represents a wholly new type of micro-organism and that it probably stands intermediate between the bacteria and protozoa as does

27. Plesset, (n. 19) *Noguchi and His Patrons*, pp. 170–171.

28. Hideyo Noguchi to L. D. Fricks, 16 October 1916, file "S. F. History (Correspondence with Noguchi—1916)," RML permanent historical files.

29. S. B. Wolbach to R. A. Cooley, 13 December 1916, volume "Professors at Various Universities," Cooley Correspondence.

30. S. Burt Wolbach, "The etiology and pathology of Rocky Mountain spotted fever: the occurrence of the parasite and the pathology of the disease in man; additional notes on the parasite (third preliminary report)," *J. Med. Res.*, *37*: 499–508, 1918.

spirochaeta."[31] The results of this work were published in 1918, in a third preliminary report, this one on spotted fever in humans.[32]

Because of his intellectually exciting discovery, Wolbach hoped to launch a large-scale research project on spotted fever at Harvard. World War I and the 1918 influenza pandemic thwarted these plans. Wolbach dropped spotted fever work for a time in order to study influenza. He wrote to Cooley: "Some day and as soon as possible we shall see an adequately organized research on Spotted Fever; but that can not be until the war is over. We are stripped to the last man here and the calls for men are so urgent that it will be impossible to put through my intention now."[33]

During that tumultuous year a tragedy in Noguchi's laboratory at the Rockefeller Institute helped to confirm Wolbach's claim that his organism was indeed the cause of spotted fever.[34] Noguchi had been hospitalized in May 1917 with typhoid fever and had suffered relapses that prevented his return to the laboratory for nearly a full year. During his absence, all of Noguchi's cultures, including those of spotted fever, were maintained by his laboratory assistant, twenty-three year old Steven Molinscek. Shortly after Noguchi's return to the laboratory in March 1918, Molinscek fell ill. On 18 March, Molinscek was hospitalized after developing a high fever and prostration. Typhoid as well as all of the diseases being studied in Noguchi's laboratory were considered in the provisional diagnosis.

Noguchi himself cultured Molinscek's blood to rule out a laboratory spirochetal infection. Typhoid was also eliminated after several Widal tests gave negative results. When Molinscek died a week later, however, the diagnosis was still uncertain. Samples of Molinscek's tissues were sent to Wolbach at Harvard for examination, and Wolbach confirmed typical spotted fever organisms in the vascular lesions. Guinea pigs inoculated with Molinscek's blood showed characteristic spotted fever signs, hence the attending physician concluded that spotted fever had been the cause.[35]

31. S. B. Wolbach to R. A. Cooley, 20 February 1918, volume "Professors at Various Universities," Cooley Correspondence.

32. Wolbach, (n. 30).

33. S. B. Wolbach to R. A. Cooley, 2 November 1918, volume "Professors at Various Universities," Cooley Correspondence.

34. My discussion of Molinscek's illness is based on Plesset's (n. 19) discussion of the accident in *Noguchi and His Patrons*, p. 173, and on his hospital report, folder "Molinscek," box 20, Record Group No. 210.3, Rockefeller University Archives, New York, New York. Hereinafter cited as Rockefeller University Archives.

35. The records on Molinscek's death also provide an interesting view of legal and societal

By accepting Wolbach's diagnosis of Molinscek's terminal illness, the Rockefeller Institute in effect affirmed Wolbach's research. Following this incident, the Harvard pathologist prepared a definitive paper on Rocky Mountain spotted fever that occupied the entire 197 pages of the November 1919 issue of the *Journal of Medical Research*.[36] In addition to presenting an exhaustive review of the literature, clinical observations, epidemiological evidence, an analysis of the life cycle of the tick vector, and a detailed description of his histological method, Wolbach expanded his discussion of the differences he had observed between the spotted fever organism and bacteria. He particularly emphasized the fact that the organism invaded the nuclei of tick cells, often "completely filling and even distending the nucleus." Noting his early reluctance to accept the intranuclear bodies as forms of the spotted fever organism, Wolbach emphasized that he now regarded them "as the most characteristic form in infected ticks." He reiterated that this phenomenon was the impetus for concluding that the agent of spotted fever indeed represented "a new form of microorganism."[37] He proposed that it be called *Dermacentroxenus rickettsi*, taking the genus name from the tick known to carry the disease and choosing the species name "in honor of Ricketts who first saw it in the blood."[38]

Although Wolbach's work seemed incontrovertible, the fact that he could not culture the organism and satisfy Koch's second postulate cast doubt on all his findings. Moreover, research on typhus, the disease spotted fever most closely resembled, did not illuminate the relationship between the two diseases. In 1916 the Brazilian researcher Henrique da Rocha Lima had described red staining, "bluntly elliptical, olive-shaped" organisms "somewhat smaller than the smallest bacteria" that had the ability to penetrate the digestive tract cells of lice and there to multiply rapidly.[39] These organisms, da Rocha Lima maintained, were the etiolog-

attitudes toward institutional responsibility for the families of people who died from laboratory-acquired infections. Under the New York labor laws in force at the time of Molinscek's death, the Rockefeller Institute had no legal liability to provide financial remuneration to his wife and daughter, both named Mary. The Institute's attorney pointed out, however, that there was a "moral obligation" to do so and failure to provide something might precipitate "attacks from persons hostile to the Institute." Consequently, the board of trustees settled a pension on Molinscek's family that was more liberal than prevailing Workmen's Compensation requirements for other deaths covered by the law. See copies of the final financial arrangement approved by members of the Executive Committee of the Board of Scientific Directors of the Rockefeller Institute dated 29 June 1918, folder "Molinscek," box 20, Record Group No. 210.3, Rockefeller University Archives.

36. S. Burt Wolbach, "Studies on Rocky Mountain spotted fever," *J. Med. Res.*, *41*: 1–197, 1919.

37. Ibid., pp. 83, 87.

38. Ibid., pp. 87.

39. Henrique da Rocha Lima's first two papers on rickettsiae were "Beobachtungen bei

ical agents of typhus, and he named them *Rickettsia prowazecki* in honor of Ricketts and Stanislaus von Prowazek, both martyrs in typhus research. Wolbach declined to classify the spotted fever organism in the same genus as da Rocha Lima's *Rickettsia*. In arguing for two different genus names, Wolbach pointed out differences in Rickett's descriptions of the two organisms.[40]

During the next two decades, similar organisms, generally called Rickettsia-bodies, were described as parasites of other arthropod hosts, including mosquitoes and bedbugs along with ticks and lice. Somewhat confusing was the fact that many of these Rickettsia-bodies were not pathogenic for the mammals on which the arthropods fed.[41] Aside from typhus and spotted fever, only one other human disease was closely linked to the presence of Rickettsia-bodies. Identified during World War I and called by several names, the most descriptive of which was "trench fever," this disease never killed but caused great loss of manpower in armies on both sides of the conflict because of its characteristic typhus-like symptoms: debilitating high fever, headache, joint pains, and rash.[42]

Other typhus-like diseases were described clinically, but few laboratory investigations of their etiological agents were pursued because of their geographical isolation. Information gained about them, however, added anecdotal evidence that these diseases comprised a special group of infectious maladies. In 1910, for example, Alfred Conor of the Pasteur Institute in Tunis reported with a colleague on a peculiar eruptive fever

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Flecktyphuslaüsen," *Arch. Schiffs. Tropen-Hyg., 20*: 17–31, 1916; and "Zur Aetiologie des Fleckfiebers," *Berl. klin. Wchnschr., 53*: 567–572, 1916. My quotation is from the latter, which is reproduced in Nicholas Hahon, ed., *Selected Papers on the Pathogenic Rickettsiae*, Cambridge, Massachusetts, Harvard University Press, 1968, pp. 74–78, quotation from pp. 76–77. Da Rocha Lima's articles on typhus are also reproduced in Henrique da Rocha Lima, *Estudos sôbre o Tifo Exantemático*, comp. Edgard de Cerqueira Falcão, commentary Otto G. Bier, São Paulo, Brazil, 1966.

40. Wolbach, (n. 36) pp. 87–88.

41. Wolbach consistently maintained that the term *Rickettsia* should be applied only to organisms that were pathogenic for humans. Other researchers, especially E. V. Cowdry of the Rockefeller Institute, argued that human pathogenicity should not be a limiting factor. See Marshal Hertig and Simeon B. Wolbach, "Studies on rickettisa-like micro-organisms in insects," *J. Med. Res., 44*: 329–374, 1924; Edmund V. Cowdry, "Rickettsiae and disease," *Arch. Pathol. Lab. Med., 2*: 59–90, 1926.

42. The three initial papers describing trench fever were H. Töpfer, "Zur Aetiologie des 'Febris Wolhynica,'" *Berl. klin. Wchnschr., 53*: 323, 1916; idem, "Der Fleckfiebererreger in der Laus," *Deutsche med. Wchnschr., 42*: 1251–1254, 1916; and idem, "Zur Ursache und Übertragung des Wolhynischen Fiebers," *München. med. Wchnschr., 63*: 1495–1496, 1916. For reviews of trench fever research written shortly after World War I see David Bruce, "Trench Fever: Final Report of the War Office Trench Fever Investigation Committee," *J. Hyg., 20*: 258–288, 1921; Richard P. Strong, *Trench Fever: Report of Commission, Medical Research Committee, American Red Cross*, Oxford, Oxford University Press, 1918; Homer F. Swift, "Trench Fever," *Arch. Intern. Med., 26*: 76–98, 1920.

in Tunisia. Its victims exhibited what Conor called "pimply lesions" and often showed evidence of small bites, which Conor thought might have been made by mosquitoes.[43] The same year, American physician Nathan E. Brill described an unknown disease with typhus-like symptoms that he had studied in 221 patients for more than a decade.[44] No arthropods of any type were connected with this illness. Because of the thoroughness with which Brill presented his case, however, "Brill's disease" immediately attracted the attention of the research community and became a catch-all designation for unknown, typhus-like symptoms.

European researchers at colonial stations in Africa and India also enriched the literature. English and Portuguese authors described a typhus-like disease in southern Africa. There was disagreement over whether ticks played a role in the transmission of this malady and whether it should be classified as Brill's disease, but all reporters agreed that newcomers to the area were the most likely victims.[45] In 1917 J. W. D. Megaw of the Indian Medical Service related his personal encounter with a fever contracted after a tick-bite near Lucknow in the Kumaon Hills of the Himalayas. Megaw maintained that this disease was similar to, if not identical with, Brill's disease.[46] He argued, in fact, that all the typhus-like diseases with the exception of typhus itself should provisionally be classified as Brill's disease. The etiological agent, he speculated, was "probably an invisible virus," which was likely "conveyed from man to man or from another animal to man by a biting insect or tick."[47]

In the Far East, typhus-like fevers were reported from the Federated Malay States, Australia, and Japan.[48] Although knowledge about those in Australia and the Malay States was limited to clinical descriptions, the disease known for centuries in Asia and called tsutsugamushi in Japan was subjected to closer scientific scrutiny. In 1810, the Japanese Hakuju Hashimoto had described a *tsutsuga*, meaning *disease*, along the tributaries

43. Alfred Conor and A. Bruch, "Une fièvre éruptive observée en Tunisie," *Bull. Soc. path. exot.*, *3*: 492–496, 1910; reprinted in Hahon, (n. 39) *Selected Papers*, pp. 47–52.

44. Nathan E. Brill, "An acute infectious disease of unknown origin: a clinical study based on 221 cases," *Am. J. Med. Sci.*, *139*: 484–502, 1910.

45. José F. Sant'Anna, "On a disease in man following tick bites and occurring in Lourenço Marques," *Parasitology*, *4*: 87–88, 1911; George H. F. Nuttall, "On symptoms following tick-bites in man," ibid., pp. 89–93; J. G. McNaught, "Paratyphoid fevers in South Africa," *J. R. Army Med. Corps*, *16*: 505–514, 1911.

46. J. W. D. Megaw, "A case of fever resembling Brill's disease," *Indian M. Gaz.*, *52*: 15–18, 1917.

47. Ibid.

48. Oliver Smithson, "Mossman fever," *J. Trop. Med. Hyg.*, *13*: 351–352, 1910; a review of reports from the Federated Malay States is in William Fletcher, "Typhus-like fevers of unknown etiology, with special reference to the Malay States," *Proc. R. Soc. Med.*, *23*: 1021–1030, 1930.

of the Shinano River. A similar disease, thought to be carried by mites, or *mushi* in Japanese, had been known at least since the sixteenth century in southern China. Laboratory investigations of tsutsugamushi began in Japan in the early 1890s when it captured the attention of Shiramiro Kitasato, who returned from his work with Robert Koch in Germany to found the Institute for Infectious Diseases in Tokyo. Kitasato believed that the disease was of protozoan etiology, but other Japanese researchers argued for bacterial causation.[49] In 1908, U.S. Army surgeons Percy M. Ashburn and Charles F. Craig concluded from a comparative study of Rocky Mountain spotted fever and tsutsugamushi that they were distinct disease entities.[50]

The Great War of 1914–18 in Europe provided the stimulus for further intensive research on epidemic typhus itself. Although typhus did not harrass the armies of western European nations, it did ravage those of Russia, Serbia, and Poland, and after the war ended, it settled with vengeance on Polish civilians. In 1919, Wolbach was invited by the League of Red Cross Societies to head a commission for further study on typhus in beleaguered Poland.[51] This provided an opportunity for him to apply to typhus the method he had employed so successfully in spotted fever research. It also allowed Wolbach to compare the organisms of the two diseases directly.

The "minute histo-pathological study" of typhus lesions in humans and in lice conducted by the commission strongly confirmed da Rocha Lima's findings. "We conclude," Wolbach wrote in the commission's report, "that *Rickettsia prowazeki* is the cause of typhus." Not only had they found "the virus of typhus and *Rickettsia prowazeki*" inseparable in infective lice, but also "bodies indistinguishable from *Rickettsia prowazeki*, demonstrable with great regularity, in the lesions of typhus in man."[52]

Wolbach also incorporated in the commission's report a summary of knowledge about Rickettsia-bodies. Although he observed that "a satisfactory definition of rickettsia is not possible at present," it was possible

49. An excellent review of the literature on tsutsugamushi disease is in Francis G. Blake et al., "Studies on tsutsugamushi disease (scrub typhus, mite-borne typhus) in New Guinea and adjacent islands: epidemiology, clinical observations, and etiology in the Dobadura area," *Am. J. Hyg.*, *41*: 243–372, 1945; see especially pp. 246–80.

50. Percy M. Ashburn and C. F. Craig, "Comparative study of tsutsugamushi disease and spotted or tick fever of Montana," *Boston Med. Surg. J.*, *159*: 749–761, 1908.

51. The report of their work is in S. Burt Wolbach, John L. Todd, and Francis W. Palfrey, *The Etiology and Pathology of Typhus*, Cambridge, Massachusetts, League of Red Cross Societies at the Harvard University Press, 1922.

52. Ibid., pp. 3, 202.

to note the properties that the organisms held in common. They all had a bacterium-like morphology but were smaller than bacteria. The difficulty of staining them with solutions used for bacteria was "a striking feature," as was "the failure to retain the stain by Gram's method." There were no motile forms. None of the rickettsiae pathogenic for humans had been successfully cultured. All had arthropod hosts, were highly specific for that host and, except for typhus, were transmitted through the eggs of the female arthropod.[53]

No general acquiescence to the view that *Rickettsiae* represented a new form of microorganism was forthcoming from the worldwide scientific community. Julius Schwalbe, Berlin correspondent for the *Journal of American Medical Association*, remarked in June 1921, that, despite numerous investigations on the etiology of typhus, there was still no common agreement. Because Rickettsia-bodies had not been cultured on artificial media, many investigators continued to reject rickettsial causation of all the typhus-like diseases and to support instead an alternative bacterial, protozoan, or viral etiology.[54] In 1920, for instance, a Brazilian researcher asserted that typhus was caused by a protozoan organism of the *Herpetomanas* genus, a group which he regarded as "piroplasms in a farther advanced stage of evolution."[55] The *Piroplasma* genus, to which he referred, were protozoan organisms, some of which caused blood diseases in cattle and dogs. Two years later, another Brazilian claimed to have cultured a different typhus organism, which he described as a bacterium, on ascitic agar, a lifeless medium.[56] H. M. Woodcock, a Fellow at University College, London, preferred to dispense entirely with the concept of disease-causing Rickettsia-bodies. He argued that they were merely the end process of cell lysis and hence the cause of the typhus-like diseases was "an abnormal haemetabolic enzyme."[57] A group of researchers at Mt. Sinai Hospital in New York compared Rickettsia-bodies with a bacillus identified in 1914 by Harry Plotz as a possible cause of typhus and concluded only that they were different. They withheld judgment on the precise relationship between typhus and either organism. Not

53. Ibid., pp. 123–124.

54. Report of Berlin correspondent, *JAMA*, *76*: 1780, 1921.

55. Abstract of D. Montfallet, "A protozoon in relation to typhus," in *JAMA*, *76*: 900, 1921.

56. This work was mentioned and rebutted in Peter K. Olitsky, "Definition of experimental typhus in guinea-pigs," *JAMA*, *78*: 571–574, 1922.

57. H. M. Woodcock, "'Rickettsia'-bodies as a result of cell-digestion or lysis," *J. R. Army Med. Corps*, *40*: 81–97, 241–269, 1923; idem, "On the modes of production of 'rickettsia'-bodies in the louse," ibid., *42*: 121–131, 175–186, 1924; quotation from this paper.

even discussing Wolbach's claim that Rickettsia-bodies were unique organisms, they focused only on the bacterial or protozoan nature of the organisms, concluding that the evidence remained insufficient to classify them as either.[58]

One "red herring" that complicated the picture further emerged from the 1916 discovery of Viennese physician Edmund Weil and his English associate Arthur Felix that a strain of *Proteus* bacillus was agglutinated by the sera of typhus patients.[59] They used this phenomenon to develop a serological test for typhus, which became known as the Weil-Felix reaction. Although subsequent studies revealed that this reaction was caused by a chance antigenic "fit" between the *Proteus* bacillus and the typhus organism, a few European bacteriologists declared that the *Proteus* bacillus was the "exciting organism" of typhus, while others argued that *Proteus* and the typhus virus were simply variants of the same organism. One champion of the latter theory was Berlin investigator Max H. Kuczynski, whose assistant, Elisabeth Brandt, died of a laboratory-acquired spotted fever infection. Kuczynski claimed to have cultured a spotted fever variant of the *Proteus* organism, but his experiments were never replicated in other laboratories.[60]

Finally, a few scientists, primarily Europeans, maintained that filterable viruses were the actual etiological agents of the typhus-like diseases. In this theory, Rickettsia-bodies were considered either coincidental or a variant form of the viral agent.[61] The agents of spotted fever and typhus

58. Leo Loewe, Saul Ritter, and George Baehr, "Cultivation of rickettsia-like bodies in typhus fever," *JAMA, 77*: 1967–1969, 1921.

59. Edmund Weil and Arthur Felix, "Zur serologischen Diagnose des Fleckfiebers," *Wien. klin. Wchnschr., 29*: 33–35, 1916; reprinted in Hahon, (n. 14) *Selected Papers on the Pathogenic Rickettsia*, pp. 79–86.

60. Report, (n. 54); B. Fejgin, "Au sujet du sérum de Kuczynski et d'une variation du Proteus X19 obtenue à partir de Rikettsia provazeki," *Compt. rend. Soc. biol., 95*: 1208–1210, 1926; Ludwik Anigstein and R. Amzel, "Researches sur l'etiologie du typhus exanthématique. Le typhus exanthéatique chez les cobazes infectés par les cultues du germe," ibid., *96*: 1502, 1927; Max H. Kuczynski and Elisabeth Brandt, "Neue atiologische und pathogenetische Untersuchungen in der 'Rickettsiengruppe,'" *Krankheitsforschung, 3*: 26–74, 1926; idem, *Die Erreger des Fleck- und Felensfiebers, Biologische und Pathogenetische Studien*, Berlin, Julius Springer, 1927; abstract of M. Ruiz Castaneda and S. Zia, "Antigenic relationship of Proteus X19 to typhus rickettsiae," *Arch. Pathol., 16*: 419, 1933. Elisabeth Brandt's death was noted in Richard Otto, "Fleckfieber und Amerikanischs Felsengebirgsfieber," *Centralbl. f. Bakteriol., 106*: 279–291, 1928.

61. For examples of this position see Frederick Breinl, "Betrachtungen, über die Immunität bei einigen Erkrankungen mit ultravisiblem Erreger," *Deutsche med. Wchnschr., 51*: 264–266, 1925; Rudolf Weigl, "Der Gegenwärtige Stand der Rickettsiaforschung," *Klin. Wchnschr., 3*: 1590–1594, 1636–1641, 1924; abstract of I. W. Hach, "Experimental typhus. IV. Filterability of virus of typhus," *Arch. Pathol. Lab. Med., 3*: 318, 1927; abstract of P. Hauduroy, "Etiology of typhus," in "Recent research on typhus," *JAMA, 85*: 1844, 1925.

had been demonstrated to be unfilterable, but that of trench fever had been filtered by the American Trench Fever Commission.[62] "Filterability," it should be noted, was one of two links among a variety of unidentified sub-microscopic agents of disease and was not an entirely precise term, since experimental conditions such as the type of filter and the pressures exerted could vary. The other link between these agents, of course, was their inability to be cultured on lifeless media.

Several investigators, including Wolbach and Rockefeller Institute researcher Peter J. Olitsky, attempted to convince their scientific associates that Rickettsia-bodies, like the filterable viruses, were obligate intracellular parasites—that is, they multiplied only within living cells. Such pathogens, they argued, would have to be grown using the emerging method of tissue culture, and they experimented with various tissue and media combinations.[63] Unfortunately, the crude tissue culture techniques then available did not support luxurious multiplication of rickettsial organisms. The limitations of technique impeded a clear demonstration that Rickettsia-bodies required the presence of living cells to multiply.

Although definitive proof eluded him, Wolbach continued to argue that the unique characteristics of Rickettsia-bodies demanded modification of Koch's postulates. Dismayed that many investigators adhered blindly to the criteria established for bacterial diseases no matter what laboratory investigations revealed, Wolbach spoke out forcefully on the issue in a 1925 speech to a meeting of the New York State Association of Public Health Laboratories. "I wish to emphasize and to insist," Wolbach stated,

> on the importance of methods which may be employed in the face of failure to cultivate insect-borne microorganisms in artificial mediums. Properly conducted experiments in which the insect vector serves as culture tube, after natural or artificial introduction of the 'virus,' have yielded evidence fully as reliable and in my opinion less open to misconstruction than in vitro cultivation. I feel it to be a duty to challenge skepticism based on rigid adherence to Koch's

62. Edmund V. Cowdry. "Rickettsiae and disease," *Arch. Pathol. Lab. Med., 2*: 59–90, 1926; reference to Trench Fever Commission on p. 63.

63. S. Burt Wolbach and M. J. Schlesinger, "The cultivation of the microorganisms of Rocky Mountain spotted fever (*Dermacentroxenus rickettsi*) and of typhus (*Rickettsia prowazeki*) in tissue plasma cultures," *J. Med. Res., 44*: 231–256, 1923; Peter K. Olitsky and James E. McCartney, "Experimental studies on the etiology of typhus fever. V. Survival of the virus in collodion sacs implanted intra-abdominally in guinea pigs," *J. Exp. Med., 38*: 691, 1928. A few years later, rickettsiae were also cultured in media formulated for the growth of viruses. See Clara Nigg and Karl Landsteiner, "Studies on cultivation of typhus fever rickettsia in presence of live tissue," ibid., 55: 563–576, 1932.

postulates when dealing with insect-borne diseases. . . . I do not know what to say to those who, in the face of the evidence I have assembled, may still insist that *Rickettsia prowazeki* is not the cause of typhus, but simply invariably accompanies the virus of typhus, particularly to those who assume . . . that the virus of typhus in man may be in ultramicroscopic form. The same line of reasoning may be applied to all infectious agents, whether or not cultivated in test tubes.[64]

The controversy over the relationship of Rickettsia-bodies to disease, however, was finally settled only as a consequence of developments in research on the filterable viruses. Wolbach himself had remarked as early as 1912 that "when our knowledge of filterable viruses is more complete, our conception of living matter will change considerably, and . . . we shall cease to attempt to classify the filterable viruses as animal or plant."[65] His views were supported by many of the leaders of virus research, notably Thomas Rivers at the Rockefeller Institute and William G. MacCullum of Johns Hopkins University Medical School. At the 1925 meeting of the American Association for the Advancement of Science, for example, MacCullum observed that progress in viral research had been slow because "we still use blindly the methods of investigation worked out for bacteriology." He suggested that "totally different mediums" might be necessary for the cultivation of viruses in addition to a conception of their nature different from existing views.[66]

The belief in all microbial pathogens as minute plants or animals, however, was slow to change. In a 1930 editorial on viruses, for example, the *Journal of the American Medical Association* observed that viruses might merely be "unusually small or unusually flaccid bacteria or protozoa," a concept that "would not introduce any new factors into current pathologic theory." There were, the *Journal* noted, bateriologists who proposed a "nonmicrobic 'liquid life'" theory and botanists who entertained the hypothesis that viruses were "self-propagating toxins, enzymes, or 'morbidic bions.'" Should either theory be correct, the editorial continued,

64. S. Burt Wolbach, "The rickettsiae and their relationship to disease," *JAMA, 84*: 723–728, 1925.

65. Quoted in Hughes, (n. 2) *The Virus*, p. 86.

66. William G. MacCullum, "A survey of our present knowledge of filterable viruses," *Arch. Pathol. Lab. Med., 1*: 487–488, 1926. An excellent view of the development of virology is offered in Saul Benison's oral history memoir of Thomas Rivers, *Tom Rivers: Reflections on a Life in Medicine and Science*, Cambridge, M.I.T. Press, 1967. On this early period, see especially chapters 3 to 6. Rivers's views on the state of knowledge regarding virus diseases were similar to those of MacCullum; see excerpts from a paper on the subject given by Rivers at a symposium sponsored by the Society of American Bacteriologists in ibid., pp. 110–111; the entire text is in T. M. Rivers, "Filterable viruses: a critical review," *Arch. Pathol. Lab. Med., 3*: 525–528, 1927.

"such transmissible biochemical perversion would necessitate radical revisions of present methods of research and clinical attack."[67]

In 1935 the need for such a radical revision in concept was demonstrated, when Wendell M. Stanley, a biochemist at the Rockefeller Institute crystallized the tobacco mosaic virus. Stanley, who later won a Nobel prize for his work, viewed the virus as an "autocatalytic protein, which, for the present, may be assumed to require the presence of living cells for multiplication."[68] Before this revolutionary discovery, viral research had focused primarily on study of the infectious diseases caused by the sub-microscopic agents. Subsequently, the techniques of the relatively new discipline of biochemistry were employed in an intense period of study that revealed the nucleic acid and protein composition of viruses—findings that rekindled discussion about the definition of life itself.[69]

During the time that this theoretical debate was raging, most researchers in the United States accepted the concept of rickettsial causation and proceeded on an empirical basis to develop vaccines against the rickettsial diseases.[70] The first successful rickettsial vaccine protected against Rocky Mountain spotted fever. Developed in 1925 from the tissues of infected ticks by Roscoe R. Spencer and Ralph R. Parker of the United States Public Health Service, it dramatically reduced the mortality from virulent spotted fever.[71]

67. "Dwarf bacteria and pigmy protozoa," *JAMA, 94*: 795–796, 1930.

68. Wendell M. Stanley, "Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus," *Science, 81*: 644–645, 1935; Stanley's work has recently been reassessed within the intellectual and social framework of the 1930s. See Lily E. Kay, "W. M. Stanley's crystallization of the tobacco mosaic virus, 1930–1940," *Isis, 77*: 450–472, 1986.

69. Earlier definitions of "life" turned on the ability of organisms to metabolize and to reproduce themselves independently. Viruses cannot perform these functions alone but must take over the genetic machinery of a functioning cell. Sally Smith Hughes observed, "With regard to the nature of viruses, biochemical findings appeared to support the idea that viruses are very large molecules, a refinement of the nonmicrobial concept of the virus. Yet it was also true that the ability of viruses to multiply and to infect were properties traditionally associated with the living state. Hence they possessed both animate and inanimate characteristics." See Hughes, (n. 2) *The Virus*, pp. 89–92.

70. In studies made after a successful vaccine was developed, for example, Roscoe R. Spencer and Ralph R. Parker found infective ticks in which rickettsiae could not be demonstrated and, conversely, noninfective ticks containing rickettsiae. Other experiments showed that the red and white blood cells of guinea pigs remained infective even after repeated washings by slow speed centrifugation, although microscopic demonstration of rickettsiae was virtually impossible. They concluded that "the virus of Rocky Mountain spotted fever may assume a form incapable of demonstration by known methods." See Parker and Spencer, "A study of the relationship between the presence of rickettsialike organisms in tick smears and the infectiveness of the same ticks," *Pub. Health Rep., 41*: 461–469, 1926; idem, "Certain characteristics of blood virus," ibid., *41*: 1817–1822, 1926; both articles reprinted in idem, "Studies on Rocky Mountain spotted fever," U. S. Hygienic Laboratory *Bulletin* no. 154 (1930), pp. 36–44, 44–49; quotation taken from this publication, p. 49.

71. For a summary of work on the spotted fever vaccine see Spencer and Parker, (n. 70) *Bulletin*.

Only during the late 1930s was the long conceptual dispute settled. Agreement that viruses and rickettsiae required living cells to multiply was fostered by the development of a different type of tissue culture technique. In 1931 Alice Miles Woodruff and Ernest Goodpasture at Vanderbilt University discovered that the chorio-allantoic membrane in the developing chick-embryo provided an ideal medium for the growth of fowl-pox virus.[72] In 1937 Herald R. Cox at the Public Health Service's Rocky Mountain Laboratory found that the yolk-sac membrane of the developing chick-embryo was similarly successful as a site for cultivating rickettsiae.[73] As the 1930s drew to a close, virtually the entire scientific community accepted the concept that viruses were obligate intracellular parasites. The debate about rickettsia was also resolved, but, to use medical parlance, it ended in "lysis" not "crisis." Contrary opinions simply ceased to be published.

For the next quarter of a century, many questions remained about the precise relationships among microbial and sub-microbial agents. Until the 1960s, for example, rickettsiae were often characterized as organisms midway between viruses and bacteria. Beginning with electron microscopy, new techniques eventually revealed that rickettsiae possessed the structure and function of true bacteria.[74] Although the precise mechanism of rickettsial pathology has yet to be elucidated completely, by the end of the 1930s the existence of pathogens different from common bacteria and protozoa was no longer in question. Koch's second postulate was modified to reflect this new consensus. *In vitro* cultivation came to include tissue-culture or chick-membrane culture of viruses and rickettsiae along with standard lifeless-media techniques for bacteria.

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72. Alice Miles Woodruff and Ernest W. Goodpasture, "The susceptibility of the chorio-allantoic membrane of chick embryos to infection with the fowl-pox virus," *Am. J. Pathol., 7*: 209–222, 1931.

73. Herald R. Cox, "Use of the yolk sac of developing chick embryo as a medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups," *Public Health Rep., 53*: 2241–2247, 1938.

74. Major studies on the structure and function of rickettsiae that led to their classification as true bacteria include Harry Plotz, Joseph E. Smadel, Thomas F. Anderson, and Leslie A. Chambers, "Morphological structure of rickettsiae," *J. Exp. Med., 77*: 355–358, 1943; Hans Ris and John P. Fox, "The cytology of rickettsiae," ibid., *89*: 681–686, 1949; Moselio Schaecter, F. M. Bozeman, and J. E. Smadel, "Study on the growth of rickettsiae: II. Morphologic observations of living rickettsiae in tissue culture cells," *Virology, 3*: 160–172, 1957; R. L. Anacker, Kazue Fukushi, E. G. Pickens, and D. B. Lackman, "Electron microscopic observations of the development of *Coxiella burnetii* in the chick yolk sac," *J. Bacteriol., 88*: 1130–1138, 1964.