

## Koch's Postulates and the Etiology of AIDS: An Historical Perspective

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**ABSTRACT** - This paper examines the debate over the human immunodeficiency virus (HIV) as the cause of acquired immunodeficiency syndrome (AIDS) from an historical perspective. The changing criteria for proving the link between putative pathological agents and diseases are discussed, beginning with Robert Koch's research on anthrax in the late nineteenth century. Various versions of 'Koch's postulates' are analyzed in relation to the necessity and sufficiency arguments of logical reasoning. In addition, alterations to Koch's postulates are delineated, specifically those required by the discovery of rickettsiae and viruses in the early twentieth century and by the immunological testing developed after midcentury to demonstrate the links between elusive viral agents and two diseases, hepatitis B and infectious mononucleosis. From this perspective, an examination of the AIDS debate is constructed. Molecular biologist Peter Duesberg's argument that HIV is not the cause of AIDS is analyzed in light of his contention that a version of Koch's postulates has not been satisfied. Additional research findings through 1990 relating to the etiology of AIDS are also noted.

When, in 1981, an unusual new condition that would become known as acquired immunodeficiency syndrome, or AIDS, was first described, nearly a century had elapsed since Robert Koch first published criteria for demonstrating that a particular bacterium caused a particular infectious disease. By 1984 investigators in France and the United States announced that AIDS was caused by a retrovirus, which is now called the Human Immunodeficiency Virus, or HIV. Diagnostic tests were prepared on the basis of this finding, and research continues on therapies for the conditions and complications of HIV infection and on a vaccine to prevent it.<sup>1</sup> The scientific consensus linking AIDS to HIV infection has been so complete, in fact,

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<sup>1</sup> At present, zidovudine, commonly known as AZT, is the principal therapy used to treat AIDS. See Linda J. Wastila and Louis Lasagna, 'The History of Zidovudine (AZT)', *Journal of Clinical Research and Pharmacoepidemiology*, 4 (1990), 25-37.

that when Peter Duesberg, an eminent molecular biologist at the University of California, Berkeley, challenged this dogma, he was initially greeted with nearly universal silence from his peers.

Medical history, of course, has resounded with debates over the etiology of disease, and many putative organisms have later been discredited. Historians might well wonder whether Duesberg's apparently singular objections have any merit, or whether he is stubbornly refusing to accept the results of rigorous investigation. Duesberg's arguments are even more interesting because they are based on the conclusion that HIV does not satisfy Koch's postulates and thus cannot be the cause of AIDS. This assertion raises anew a very old question: 'How do investigators prove the link between a putative pathological agent and a disease?' In this paper, I will examine how scientists in the past have marshalled proof for particular disease agents, and I will utilize this historical perspective to illuminate the arguments in the debate over the AIDS agent.

#### *Background: Koch's Postulates and Koch's Own Research*

Let us begin with Robert Koch himself, who was born December 11, 1843, the son of a mining engineer.<sup>2</sup> Koch studied medicine at the University of Göttingen, where he was influenced by Jacob Henle, the professor of anatomy who had published in 1840 on the possible relationship of microorganisms to disease and whose name is often linked with Koch's in discussions of the postulates.<sup>3</sup> After receiving his M.D. degree in 1866, Koch spent several unsettled years attempting to establish a general practice. In 1870 he volunteered for service in the Franco-Prussian War and gained experience with typhoid and wound infections that proved invaluable in his later research. From 1872 to 1880 he served as District Medical Officer for Wollstein, a position in which he was able to pursue the research on anthrax and on wound infections that catapulted him to prominence.

In Koch's research on anthrax, he struggled for the first time with the problem of proving causality. He knew, for example, that ingesting anthrax bacilli did not always result in disease, so he could not argue that in every case the presence of anthrax bacilli would cause the

<sup>2</sup> An excellent recent biography of Koch is Thomas D. Brock, *Robert Koch: A Life in Medicine and Bacteriology*, Madison, Wis., Science Tech Publishers; Berlin: Springer-Verlag, 1988.

<sup>3</sup> Henle emphasized the need to isolate organisms to see if they might cause diseases, an achievement that was impossible with the laboratory methods available in 1840. See R. N. Doetsch, 'Henle and Koch's Postulates', *ASM News*, 48 (1982), 555-56.

disease. His 1876 paper, which claimed to link *Bacillus anthracis* irrefutably with the disease, relied on absence arguments: if the bacilli were not present, no disease occurred.<sup>4</sup> Koch's work was not accepted without challenge. His critics demanded that the putative anthrax bacillus be separated from any other substance that might potentially cause the disease. Because Koch himself still used liquid culture techniques at that time, he replied that such proof would be impossible.

Two years later, in 1878, Koch published two papers on wound infections<sup>5</sup>. He utilized similar absence arguments as the basis for proving bacterial etiology. In the second paper, Koch laid out for the first time his specific criteria for establishing causation – the first statement of his postulates. They were:

1. The microorganism must be exhibited in all cases of the disease.
2. The distribution of the microorganism must correlate with and explain the disease phenomena.
3. For each different disease, a morphologically distinguishable microorganism must be identified.

In commenting on these postulates, I shall begin with the last, which caused Koch some problem. Morphologically indistinguishable microorganisms occasionally produced different effects, such as different toxins. Since these differences could be identified by means of biochemical or other methods, Koch eventually modified this criterion to the following form: 'The microorganism must be distinguishable in some way from organisms that are associated with other diseases'. The second point in this version of Koch's postulates relates to clinical manifestations of the infection, and serves to reinforce the first point, which clearly reflects Koch's reasoning about causality. It does not claim that the presence of a microorganism will invariably cause a disease, but rather that in its absence no disease occurs.

Koch's philosophical position in this early statement of his postulates, as well as in their later evolution, has been examined by K. Codell Carter, professor of philosophy at Brigham Young University. To analyze Koch's reasoning, Carter employs two rules of logic, necessity and sufficiency, which are key concepts in proving causality in infectious diseases: 1) a phenomenon C is *sufficient* for a phenomenon

<sup>4</sup> Robert Koch, 'Die Ätiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des Bacillus Anthracis', In: J. Schwalbe, (ed.), *Gesammelte Werke von Robert Koch*, Leipzig: Georg Thieme, 1912, 2 vols. 1: 5-25. An English translation is in K. Codell Carter, trans., *Essays of Robert Koch* New York: Greenwood Press, 1987, pp. 1-18.

<sup>5</sup> Robert Koch, 'Neue Untersuchungen über die Mikroorganismen bei infektiösen Wundkrankheiten', In: Schwalbe, (ed.), *Gesammelte Werke von Robert Koch*, 1: 57-60; idem, 'Untersuchungen über die Ätiologie der Wundinfektionskrankheiten', *ibid.*, pp. 61-108. An English translation of the second paper is in Carter, trans., *Essays of Robert Koch*, pp. 19-56.

E if the occurrence of C ensures the occurrence of E; and 2) a phenomenon C is *necessary* for a phenomenon E if the nonoccurrence of C ensures the nonoccurrence of E. Koch's arguments in the first statement of his postulates, Carter observed, rely on necessary but not sufficient proof.<sup>6</sup>

In 1883, Koch traveled to Egypt to investigate the etiology of cholera because there were no cholera cases in Germany from which he could derive material. Since conducting his wound infection work, Koch had developed the technique of culturing bacteria on solid media rather than in liquid, hence he was able to culture the suspect organism in what came to be called 'pure culture'. Was this vibrio, which he considered distinct from others of similar morphology, the cause of cholera, or was it perhaps normally present in the intestinal lining and multiplied only when another pathological process, which would be the 'true' cause of cholera, provided the opportunity?

Unfortunately, cholera is a disease exclusively of humans, so he was unable to study it in experimental animals.<sup>7</sup> Because of this limitation, as William Coleman has pointed out, Koch's reasoning about the comma-shaped *Vibrio cholera* had to be based on interpretation of epidemiological data.<sup>8</sup> With his associates, Koch identified the organism in every case of cholera that he studied. Of equal importance, Koch found no cholera vibrios in healthy persons or in persons with different diseases. He rested his case on a strong necessity argument – without the organism, there was no disease – and on a weak sufficiency argument – if the disease was present, then the organism was also present.

Concomitantly with the work on cholera, Koch investigated the etiology of tuberculosis. In 1882 he announced the discovery of a bacillus as the cause of the White Plague.<sup>9</sup> Because tuberculosis was arguably the most dread disease of the nineteenth century, Koch's

<sup>6</sup> K. Codell Carter, 'Koch's Postulates in Relation to the Work of Jacob Henle and Edwin Klebs', *Medical History*, 29 (1985), 353-74; logical criteria stated on p. 354.

<sup>7</sup> Dogs and guinea pigs were eventually infected with cholera, but only when their bile ducts were tied off and a pure cholera culture injected directly into the duodenum. See Brock, *Robert Koch*, pp. 180-82.

<sup>8</sup> William Coleman, 'Koch's Comma Bacillus: the First Year', *Bulletin of the History of Medicine*, 61 (1987), 315-42; Robert Koch, 'Erste Konferenz zur Erörterung der Cholerafrage am 26. VII. 1884 zu Berlin', in: Schwalbe, (ed.), *Gesammelte Werke von Robert Koch*, 2 (part 1): 20-60; idem, 'Über die Choleraabakterien', *ibid.*, 61-68. English translations of both papers are in Carter, trans., *Essays of Robert Koch*, pp. 151-70, 171-78.

<sup>9</sup> Koch published two papers in 1882 on tuberculosis. They are 'Die Aetiologie der Tuberkulose', in: Schwalbe, (ed.), *Gesammelte Werke von Robert Koch*, 1: 428-445, and 'Über die Ätiologie der Tuberkulose', *ibid.*, pp. 446-53. An English translation of the first paper is in Carter, trans., *Essays of Robert Koch*, pp. 83-96.

announcement that it was caused by a microorganism hastened the acceptance of the germ theory of disease as no other discovery might have done. In his work on tuberculosis, Koch was aided both by his pure culture technique and by the availability of an experimental animal. He could thus utilize necessity and sufficiency arguments in linking the organism to the disease. No case of tuberculosis, he asserted, occurred in the absence of the tubercle bacillus, and if the bacillus were properly injected into an experimental animal, it reproduced the disease in the new host.

In 1884 Koch published a monumental paper on the etiology of tuberculosis.<sup>10</sup> It is from this paper that the statement of Koch's postulates is usually drawn. He said:

1. An alien structure must be exhibited in all cases of the disease.
2. The structure must be shown to be a living organism and must be distinguishable from all other microorganisms.
3. The distribution of microorganisms must correlate with and explain the disease phenomena.
4. The microorganism must be cultivated outside the diseased animal and isolated from all disease products which could be causally significant.
5. The pure isolated microorganism must be inoculated into test animals and these animals must then display the same symptoms as the original diseased animal.<sup>11</sup>

Points 1, 2, and 3 are restatements of his earlier work. What is new here are points four and five, which Koch considered most important in his tuberculosis work and which later came to be seen as the key postulates. These two points apparently fulfill the sufficiency argument: in the presence of a particular organism, disease always follows. Yet Koch knew well that this was not always the case experimentally. Unless specific pathways of inoculation were used, the injected bacteria might not cause disease, and tubercle bacilli were widely known to

<sup>10</sup> Robert Koch, 'Die Aetiologie der Tuberkulose', In: Schwalbe, *Gesammelte Werke von Robert Koch*, 1: 467-565. English excerpts from this paper are in Carter, trans., *Essays of Robert Koch*, pp. 129-50; the entire text in English is in idem, *The Aetiology of Tuberculosis*, trans. Dr. and Mrs. Max Pinner (New York: National Tuberculosis Association, 1922).

<sup>11</sup> Carter, 'Koch's Postulates in Relation to the Work of Jacob Henle and Edwin Klebs', p. 361. Thomas D. Brock notes that a similar form of these postulates, was first published in 1883 by Koch's assistant, Friedrich Loeffler, who also became a major figure in bacteriology, in a paper on diphtheria. Brock translates the relevant passage as: 'If diphtheria is a disease caused by a microorganism, it is essential that three postulates be fulfilled. The fulfillment of these postulates is necessary in order to demonstrate strictly the parasitic nature of a disease: 1) The organism must be shown to be constantly present in characteristic form and arrangement in the diseased tissue; 2) The organism which, from its behavior appears to be responsible for the disease, must be isolated and grown in pure culture; 3) The pure culture must be shown to induce the disease experimentally'. See Brock, p. 180.



be able to exist in a host without causing disease.<sup>12</sup> Thus although these two postulates came to have a central role in later research, Koch scholars such as Carter have emphasized that Koch himself continued to rely on necessity arguments. In an address to the Tenth International Congress of Medicine in 1890, for example, Koch acknowledged that for several diseases, including typhoid fever, diphtheria, leprosy, and cholera, it had proved impossible to infect experimental animals with pure cultures and thus demonstrate the sufficiency argument. 'Therefore,' he stated, 'we are justified in stating that if . . . the regular and exclusive occurrence of the parasite is demonstrated, the causal relationship between parasite and disease is validly established'.<sup>13</sup>

### *Rickettsial Diseases and the Laboratory Cultivation of Organisms*

Just prior to Koch's death in 1910, an apparently new genus of organisms had been identified. It was eventually named *Rickettsia* after Howard Taylor Ricketts of the University of Chicago, who studied two of the diseases caused by species of rickettsiae, Rocky Mountain spotted fever and epidemic typhus. These organisms were large enough to be seen under a microscope, appeared consistently in cases of disease, and could be transmitted in blood from sick to healthy experimental animals. They could not, however, be cultured on lifeless media, and failure to meet this postulate retarded their acceptance as agents of disease.<sup>14</sup>

Between 1906 and 1908, Ricketts studied the organism that he found consistently in the blood of victims of Rocky Mountain spotted fever. By January 1909 he ventured publication of a paper in the *Journal of the American Medical Association* conservatively entitled 'A Micro-Organism Which Apparently Has a Specific Relationship to Rocky Mountain Spotted Fever'. He argued that, in addition to the presence of the organism in cases of the disease, agglutination experiments were so convincing that there must be 'a specific relationship

<sup>12</sup> Carter, 'Koch's Postulates', pp. 361-62. Koch did all of this work without knowledge of human carriers of disease - people who were well themselves but who harbored pathogenic bacteria and transmitted them to others. The human carrier was not identified until about 1893. See *ibid.*, p. 358, n. 18.

<sup>13</sup> Robert Koch, 'Über bakteriologische Forschung', In: Schwalbe, (ed.), *Gesammelte Werke von Robert Koch*, 1: 650-660; English translation of quotation cited in Thomas Rivers, 'Viruses and Koch's Postulates', *Journal of Bacteriology*, 33 (1937), 1-12; quotation from p. 4.

<sup>14</sup> Victoria A. Harden, 'Koch's Postulates and the Etiology of Rickettsial Diseases', *Journal of the History of Medicine and Allied Sciences*, 42 (1987), 277-95.

between the organism and the disease'.<sup>15</sup> While cautious in his published statements, Ricketts to his friends revealed complete assurance. 'Just a note to tell you that I have found the microorganism of spotted fever', Ricketts wrote in a letter just before the *JAMA* article was published.<sup>16</sup> Yet Ricketts was well aware that many in the scientific community would remain skeptical until the organism had been cultured on lifeless media according to Koch's postulates. 'I remind you,' he wrote to the Secretary of the Montana State Board of Health, 'that we have not yet been able to cultivate . . . [the microbe], and thus meet one of Koch's great laws. This makes it necessary to bring all kinds of indirect evidence to bear showing that we have the real thing'.<sup>17</sup> Ricketts did not live to see his work vindicated. In December 1909 he traveled to Mexico City to study typhus, became infected, and died in May 1910.

In 1916, Simeon Burt Wolbach, a pathologist at Harvard University Medical School, took up the study of rickettsiae. After working for three years with Rocky Mountain spotted fever organisms, he traveled to Poland to study epidemic typhus. His exhaustive histopathological studies revealed rickettsial organisms in the vascular lesions of victims of both diseases. Relying on the necessity argument – that without these organisms, no disease occurred – Wolbach concluded that rickettsiae were the cause of these diseases. Many investigators, however, refused to accept his conclusions because he could not culture the organisms on lifeless media.<sup>18</sup>

### *Polio Research, Filterable Viruses, and Rivers's Postulates*

During the 1920s, Wolbach was joined by many distinguished virologists, such as Thomas Rivers of the Rockefeller Institute for Medical Research, in arguing that rickettsiae and the filterable viruses were

<sup>15</sup> Howard Taylor Ricketts, 'A Micro-Organism Which Apparently Has a Specific Relationship to Rocky Mountain Spotted Fever: a Preliminary Report', *Journal of the American Medical Association*, 52 (1909), 379-80.

<sup>16</sup> Ricketts to Thomas D. Tuttle, January 25, 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.

<sup>17</sup> Ricketts to Thomas D. Tuttle, June 24, 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.

<sup>18</sup> S. Burt Wolbach, 'Studies on Rocky Mountain Spotted Fever', *Journal of Medical Research*, 41 (1919), 1-197; League of Red Cross Societies, Typhus Research Commission to Poland, *The Etiology and Pathology of Typhus*, by S. Burt Wolbach, John L. Todd, and Francis W. Palfrey (Cambridge, Mass: League of Red Cross Societies at the Harvard University Press, 1922).

obligate intracellular parasites.<sup>19</sup> Such pathogens would have to be grown using the emerging method of tissue culture, and they experimented with various tissue and media combinations. Unfortunately, the crude tissue culture techniques then available did not support luxuriant multiplication of many organisms. In 1931, however, Alice Miles Woodruff and Ernest Goodpasture at Vanderbilt University demonstrated that the chorioallantoic membrane of the developing chick embryo provided an ideal medium for the growth of fowlpox virus.<sup>20</sup> Soon other researchers identified a number of viruses that flourished on this membrane. Advances in immunology, such as the complement fixation test, also produced more reliable serological tests for the presence of antibodies to specific organisms.

Rivers also argued that alterations in Koch's postulates were necessary to establish the etiology of viral diseases. To illustrate the folly that uncritical adherence to Koch's postulates could produce, Rivers examined the claim of some investigators that streptococci were the etiological agents of poliomyelitis. Streptococci had been found associated with polio, these scientists stated; these organisms could be obtained in pure cultures from patients ill with polio; they produced paralysis when injected into monkeys and rabbits; they could again be recovered from these experimental animals; and individuals recovering from polio often possessed antibodies against streptococci. Yet physicians and scientists who were familiar with polio knew that the pathological picture of polio was different from that of streptococcal infection and that paralysis could be caused by a number of different organisms.

Rivers asserted that only two rules of proof were needed to establish etiology for viral diseases:

1. A specific virus must be found associated with a disease with a degree of regularity.

<sup>19</sup> See, for example, T. M. Rivers, 'Filterable Viruses: A Critical Review', *Archives of Pathology and Laboratory Medicine*, 3 (1927), 525-28; W. G. MacCullum, 'A Survey of Our Present Knowledge of Filterable Viruses', *ibid.* 1 (1926), 487-88; S. Bart Wolbach, 'The Rickettsiae and Their Relationship to Disease', *JAMA*, 84 (1925), 723-28. On the history of the concept of the virus and of the discipline of virology, see Sally Smith Hughes, *The Virus: A History of the Concept* (New York: Science History Publications, 1977); Saul Benison, *Tom Rivers: Reflections on a Life in Medicine and Science* (Cambridge: M.I.T. Press, 1967), esp. chs. 3-6.

<sup>20</sup> Alice Miles Woodruff and Ernest W. Goodpasture, 'The Susceptibility of the Chorio-Allantoic Membrane of Chick Embryos to Infection with the Fowl-Pox Virus', *American Journal of Pathology*, 7 (1931), 209-22. For a chronology of major papers on cultivation of other organisms by this method, see B. John Buddingh, 'Chick-Embryo Techniques', in: Thomas M. Rivers, (ed.), *Viral and Rickettsial Infections of Man*, 2nd ed. (Philadelphia: J. B. Lippincott Co., 1952), pp. 109-25; esp. table 8, p. 110.

<sup>21</sup> Rivers, 'Viruses and Koch's Postulates', pp. 5-6.



2. The virus must be shown to occur in the sick individual not as an incidental or accidental finding but as the cause of the disease under investigation.<sup>22</sup>

These criteria clearly are in line with Koch's arguments of necessity, and Rivers noted the similarity. He proceeded to make plain, however, the specific differences between Koch's rules for bacteriology and his own for viruses. A virus, for example, might not be found in every case of a disease at all times, but it 'should always be found at the proper time in specific lesions'.<sup>23</sup> Cultivation of viruses in tissue cultures was a process similar to cultivation of bacteria on lifeless media, Rivers suggested, but not an identical one. Furthermore, not all viruses could be cultured, yet this did not invalidate them as etiological agents, hence he omitted such a rule from his own proof. Although Rivers's postulates were only two in number, they required exceptional rigor by an investigator, for the possibilities of error in satisfying the second seemed endless. Rivers cited numerous instances in which investigators had been misled by preexisting or concomitant infections in experimental animals and by contamination of laboratory cultures. Rivers concluded his essay with a call for continued work by virologists, 'tempered by the priceless attributes of common sense, proper training and sound reasoning'.<sup>24</sup>

### *Minor Illnesses, Laboratory Technique, and Huebner's Guidelines*

During the 1940s, the electron microscope rendered viruses visible. Improved tissue culture techniques, the breeding of specialized strains of experimental mice, and additional immunological tests further enhanced the laboratory detection and isolation of viruses. By the 1950s, a plethora of viruses had been identified, some of which were linked with diseases. Many of them, however, remained 'viruses in

<sup>22</sup> *Ibid.*, p. 6. Although Rivers stipulated only two postulates in this paper, his followers later expanded his criteria somewhat. In the 1948 edition of his textbook on bacteriology, Martin Frobisher, Jr., summarized Rivers's postulates as follows:

1. The virus must be present in the host cells showing the specific lesions, at the time of the disease, or in the blood or other body fluids.
2. Filtrates of the infectious material (blood, etc., or tissue triturates) shown not to contain bacteria or other visible or cultivable organisms, must produce the disease or its counterpart in appropriate animals or plants.
3. Similar filtrates from such animals or plants must transmit the disease.

See Martin Frobisher, Jr., *Fundamentals of Bacteriology* 4th ed. (Philadelphia: W. B. Saunders, 1949), p. 811.

<sup>23</sup> Rivers, 'Viruses and Koch's Postulates', p. 6. Rivers also recognized the existence of healthy virus carriers.

<sup>24</sup> Rivers, 'Viruses and Koch's Postulates', p. 11.

search of disease', as the title of a 1956 conference on research in virology termed them.<sup>25</sup>

Robert J. Huebner of the U.S. National Institutes of Health, who organized this conference, observed that 'new techniques, developed to a high degree of efficiency in the last few years, have reduced the isolation of new human and animal viruses from a technological feat of high order to an almost exasperatingly commonplace occurrence'. Despite such discoveries, however, an impasse had not yet been resolved. While clinicians 'seldom if ever' knew whether a respiratory or enteric illness was actually caused by a virus, or, if so, what virus was implicated, conversely, virologists reporting new agents rarely had any inkling of their clinical behavior or importance.<sup>26</sup>

These myriad viral agents were generally characterized as causing 'minor illnesses'. Because of their wide dissemination and ease of demonstration in the laboratory, they were easy to identify, and, because virologists usually studied sick people, the viruses were obviously found in 'highly suspicious circumstances'.<sup>27</sup>

Huebner noted that careful attention to the spirit of Koch's and Rivers's postulates should prevent misidentification of these agents, but he believed that additional guidelines might be helpful. He thus proposed a nine-point program that stressed the importance of fully characterizing any new virus in the laboratory and emphasized the role of epidemiologic and serologic studies.<sup>28</sup> Furthermore, he recog-

<sup>25</sup> The conference *Viruses in Search of Disease* was sponsored by the New York Academy of Sciences, Section on Biology, 24-25 May 1956. Papers from it were published in *Annals of the New York Academy of Sciences*, 67 (1957), 209-446.

<sup>26</sup> Robert J. Huebner, 'The Virologist's Dilemma', *Annals of the New York Academy of Sciences*, 67 (1957), 430-38; discussion following, pp. 439-45; quotations from p. 430.

<sup>27</sup> *Ibid.*, p. 434.

<sup>28</sup> Huebner's guidelines may be paraphrased as follows:

1. The virus must be established as a real entity that can be cultured in other laboratories.
2. The virus must be shown to be of human origin and not a contaminant or a virus from experimental animals.
3. The virus must be shown to produce an active infection by evoking an increase in serologically demonstrable antibodies.
4. The virus should be characterized early, so that comparisons can be made as promptly as possible with other agents already described or soon to be discovered.
5. The virus should be constantly associated with a specific illness.
6. Double-blind studies of the virus in human volunteers should be made, with proper adjustments for the subjective impressions produced in both observers and subjects. This is of the utmost importance when studying poorly defined minor illnesses that are, nevertheless, familiar to all.
7. Carefully conceived epidemiologic studies coordinated with adequate laboratory and clinical observations are indispensable for the purpose of finally establishing the etiologic role of highly prevalent viruses in human disease. Two types of studies are generally employed:
  - a. Studies of populations experiencing a disease outbreak
  - b. Long term studies of community or institutional groups
8. Prevention by specific vaccination. If a vaccine prepared from a suspect virus prevents a specific disease, the virus may be said to cause the disease.

nized that several viruses might cause similar symptoms and that, for some illnesses, two or more viruses at once might be responsible.<sup>29</sup> Development of a vaccine that would prevent a specific disease, he suggested, would demonstrate etiology in a most convincing way. In this paper, Huebner introduced no new criteria. His suggestions were elaborations and refinements of Rivers's postulates that incorporated the new technologies developed since the 1930s.

### *Hepatitis, Infectious Mononucleosis, and Seroepidemiological Proof*

Huebner's guidelines recognized the importance of epidemiological data and clinical studies in establishing the association of a virus with a disease, and they underscored Rivers's observation about the importance of immunological tests. They were constructed, however, to assist virologists who had no trouble cultivating viruses in the laboratory. In contrast, the agents responsible for hepatitis and for infectious mononucleosis resisted laboratory culture for decades. Their identification, moreover, incorporated an entirely new way of demonstrating etiology.

By the end of World War II, epidemiological and clinical studies in human volunteers had confirmed that there were two forms of hepatitis, designated A and B, but their causal agents remained unknown.<sup>30</sup> So, too, the cause of infectious mononucleosis, sometimes called

9. Financial support for adequately trained technical help and for epidemiological, clinical, and laboratory research.

See *ibid.*, pp. 435-37.

<sup>29</sup> *Ibid.*, pp. 431, 434. Alfred Evans codified these ideas into a table entitled 'The Five Realities of Acute Respiratory Disease':

1. The same clinical syndrome may be produced by a variety of agents.
2. The same etiologic agent may produce a variety of clinical syndromes.
3. The predominating agent in a given clinical syndrome may vary according to the age group involved, the year, the geographic location, and the type of population (military or civilian).
4. Diagnosis of the etiologic agent is frequently impossible on the basis of the clinical findings alone.
5. The cause (or causes) of a large percentage of common infectious disease syndromes is still unknown.

See Alfred S. Evans, 'Causation and Disease: the Henle-Koch Postulates Revisited', *Yale Journal of Biology and Medicine*, 49 (1976), 175-95; this list from table 4, p. 181.

<sup>30</sup> There is a large literature on hepatitis. Overviews used for this discussion included W. Paul Havens, Jr., 'Infectious Hepatitis', *Medicine*, 27 (1948), 279-326; Frank W. Hartman, Gerald A. LoGrippe, John G. Mateer, and James Barron, (eds.), *Hepatitis Frontiers* (Boston: Little, Brown, 1957), a symposium sponsored by the Henry Ford Hospital, Detroit, Michigan, 25-27 October 1956; Joseph L. Melnick, Gordon R. Dreesman, and F. Blaine Hollinger, 'Viral Hepatitis', *Scientific American*, 237 (July 1977), 44-52; A. J. Zuckerman, *Human Viral Hepatitis: Hepatitis-Associated Antigen and Viruses*, 2nd ed. rev. (New York: Elsevier, 1975) Irving Millman, Toby K. Eisenstein, and Baruch S. Blumberg, (eds.), *Hepatitis B: The Virus, the Disease, and the Vaccine*, proceedings of a symposium held 11-12 November 1982 in Philadelphia, Pennsylvania, New York: Plenum Press, 1984.

'glandular fever' eluded detection.<sup>31</sup> Although epidemiological evidence pointed to viral etiologies, investigators in the 1950s and early 1960s could neither grow the presumed viruses in cultures nor identify them with the electron microscope.

In 1964, M. A. Epstein, Y. M. Barr, and B. G. Achong demonstrated herpes-like particles under the electron microscope in tumor cells cultured from a patient with what is now called Burkitt's lymphoma. The following year, Baruch Blumberg of the Fox Chase Cancer Center in Philadelphia, Pennsylvania, published his discovery of a new antigen in the serum of an Australian aborigine. Neither of these agents had been linked with any infectious disease, but recently developed immunological tests administered in large-scale epidemiological studies eventually demonstrated that they had a causal relation to infectious mononucleosis and hepatitis B, respectively.<sup>32</sup> Furthermore, with the advent of genetic engineering, the hepatitis B surface antigen could be cloned to produce an effective vaccine. This achievement would certainly have satisfied Huebner's guideline that an effective preventative would link an agent etiologically to a disease, even in the absence of virus identification.

Such immunological and epidemiological techniques have strongly influenced the thinking of the most recent generation of scientists. In another key paper examining methods of scientific proof, Alfred S. Evans of Yale University outlined the method for demonstrating causation of disease by immunological methods. His criteria are:

1. Antibody to the agent is regularly absent prior to the disease and to exposure to the agent (i.e., before the incubation period).
2. Antibody to the agent regularly appears during illness and includes both IgG- and IgM-type antibodies.
3. The presence of antibody to the agent indicates immunity to the clinical disease associated with primary infection by the agent.
4. The absence of antibody to the agent indicates susceptibility to both infection and the disease produced by the agent.
5. Antibody to no other agent should be similarly associated with the disease unless it is a cofactor in its production.<sup>33</sup>

Points 1 and 4 in this scheme restate in immunological terms Koch's first postulate, that without the virus, no disease exists. Point 5 notes that only one virus must be associated with the disease to be

<sup>31</sup> On infectious mononucleosis, see R. S. Chang, *Infectious Mononucleosis* (Boston: Hall, 1980) David Schlossberg, (ed.), *Infectious Mononucleosis*, 2nd ed. New York: Springer-Verlag, 1989.

<sup>32</sup> See Evans's discussion of this in 'Causation and Disease', pp. 182-84.

<sup>33</sup> *Ibid.*, p. 184.

termed etiological, unless, of course, two or more viruses act together to produce the disease. Points 2 and 3 reflect the actual sequence observed in hepatitis and in infectious mononucleosis, but these two 'postulates' are not applicable to all infectious diseases.

### *Subviral Agents and Koch's Postulates*

The recognition of two subviral agents, one accepted and another under investigation, should be mentioned briefly, because they emphasize the fact that Nature continues to surprise us with pathogens that may require an alteration in methodology to demonstrate their existence. The first is the viroid, essentially a free nucleic acid without the characteristic virus-like protein coat, which has been identified as the pathogen responsible for several diseases of plants. During the 1960s, T. O. Diener of the University of Maryland and the U.S. Department of Agriculture established that a viroid was the cause of potato spindle tuber disease. Subsequently, viroids have also been linked to other plant diseases.<sup>34</sup> In studying the etiology of plant diseases, immunological methods like those used in hepatitis research are not applicable, because plants have no immune systems comparable to those of animals.

Koch's postulates have been adapted, however, for research on the etiology of plant diseases. This set of criteria was prepared by L. Bos of the Research Institute for Plant Protection in Wageningen, the Netherlands:

1. The virus must be concomitant with the disease.
2. It must be isolated from the diseased plant:
  - separated from contaminating pathogens
  - multiplied in a propagation host
  - isolated from plant sap and purified physicochemically
  - identified for its intrinsic properties.
3. When inoculated into a healthy host plant, it must reproduce the disease.
4. The same virus must be demonstrated to occur in and must be re-isolated from the experimental host.<sup>35</sup>

<sup>34</sup> T. O. Diener, 'The Viroid'. In: Frank Fenner and Adrian J. Gibbs, (eds.), *Portraits of Viruses: A History of Virology*, Basel: Karger, 1988, pp. 189-204; idem, (ed.), *The Viroids*, New York: Plenum Press, 1987.

<sup>35</sup> L. Bos, 'Hundred Years of Koch's Postulates and the History of Etiology in Plant Virus Research', *Netherlands Journal of Plant Pathology*, 87 (1981), 91-110; esp. table 4, p. 101.



Satisfaction of point two, argues T. O. Diener, is 'much more complex' than fulfillment of Koch's original criterion that bacteria be grown in pure laboratory culture.<sup>36</sup> The methods of molecular biology, however, have enhanced physical and chemical characterization of both plant and animal pathogens.

The second, a putative subviral agent, is the unconventional slow virus, sometimes called a prion. Described as the antithesis of the viroid, this pathogen has been defined as an infectious particle comprised of protein with no identifiable nucleic acid.<sup>37</sup> An unconventional slow virus was first proposed as the agent responsible for kuru, a fatal disease found in New Guinea, by D. Carleton Gajdusek.<sup>38</sup> Slow viruses have also been linked to Creutzfeldt-Jakob disease, Gerstmann-Sträussler syndrome, and several fatal diseases in animals. These agents, which affect the central nervous system, have long incubation periods and elicit no immunological response from their hosts – thus making serological studies as useless as in plant diseases. They resist treatment with standard agents that kill viruses and bacteria and are always fatal. As yet, this class of pathogen has not been fully characterized. In 1974, however, Richard T. Johnson of the Johns Hopkins University School of Medicine and C. J. Gibbs, Jr., of the National Institutes of Health, took on the task of formulating criteria for establishing etiology in unconventional slow viral infections. The criteria they developed are:

1. There should be consistency in the transmission of the disease to experimental animals or some consistency in the recovery of the virus in cell cultures, and this transmission or recovery should be confirmed by more than one laboratory.
2. Either serial transmission of the clinicopathologic process should be accomplished using filtered material and serial dilutions to establish replication of the agent, or the recoverable agent should be demonstrated with consistency in the diseased tissue by electron microscopic, immunofluorescent, or other methods, and should be demonstrated in the appropriate cells to explain the lesions.

<sup>36</sup> T. O. Diener to Victoria A. Harden, February 5, 1990, file 'History of Virology Course, 1990, Correspondence', NIH Historical Office. I am grateful to Dr. Diener for his comments and suggested references.

<sup>37</sup> On slow virus diseases and the prion, see Richard T. Johnson, 'Chronic Infectious Neuropathic Agents: Possible Mechanisms of Pathogenesis', *Current Topics in Microbiology and Immunology*, 40 (1967), 3-8; Stanley B. Prusiner and Michael P. McKinley, (eds.), *Prions: Novel Infectious Pathogens Causing Scrapie and Creutzfeldt-Jakob Disease* (San Diego, Calif: Academic Press, 1987); Katsumi Dohura; Jun Tateishi; Tetsuyuki Kitamoto; Hiroyuki Sasaki; and Yoshiyuki Sakaki, 'Creutzfeldt-Jacob disease patients with Congoophilic Kuru Plaques have the Missense Variant Prion Protein Common to Gerstmann-Sträussler Syndrome', *Annals of Neurology*, 27 (1990), 121-26.

<sup>38</sup> D. Carleton Gajdusek, 'Unconventional Viruses and the Origin and Disappearance of Kuru', Nobel lecture reprinted in *Science*, 197 (1977), 943-60; esp. table 3, p. 944.

3. Parallel studies of normal tissues or tissues of patients with other diseases should be carried out to establish that the agent is not an ubiquitous agent or a contaminant.<sup>39</sup>

Johnson and Gibbs's criteria are reminiscent of Rivers's in their brevity, their generality, and their demands for rigor. Having established the necessity argument, that without the agent, no disease occurs, they seek to fulfill the sufficiency argument that would characterize a specific entity and demonstrate that it will induce the disease.

### *Koch's Postulates and HIV as the Cause of AIDS*

The central purpose of the various versions of Koch's postulates, their modifications, and alternative criteria for establishing disease etiology has been to provide mechanisms by which the logical arguments of necessity and sufficiency could be satisfied. As sophistication in laboratory experimentation has increased, some particulars of these rules have changed. The characteristics of specific agents under investigation, furthermore, have also required alterations in method. Those investigators most successful in elucidating the etiology of new diseases have grasped the philosophical essence of Koch's postulates and have shaped or reshaped their techniques toward solving a particular problem. Thus Ricketts and Wolbach did not feel hampered by the fact that rickettsiae would not grow on lifeless media; Rivers emphasized that poliovirus was the cause of polio even if it could not be isolated from every case at any given time; Blumberg realized that the Australia antigen could serve as an indicator of hepatitis B infection even though no virus had been isolated; and Gadjusek recognized that kuru was the result of an infectious process even though no typical symptoms of infection were present. With these points in mind, let us examine the debate over the Human Immunodeficiency Virus as the cause of AIDS.

Peter H. Duesberg is noted for his research on viral oncogenes. He is a member of the prestigious National Academy of Sciences in the United States. Duesberg published his original criticism of HIV in 1987 in a paper entitled 'Retroviruses as Carcinogens and Pathogens: Expectations and Reality.' The title itself indicates that Duesberg's arguments are not limited to the AIDS agent but encompass all retroviruses that do not possess transforming onc, or cancer causing, genes.

<sup>39</sup> R. T. Johnson and C. J. Gibbs, Jr., 'Editorial: Koch's Postulates and Slow Infections of the Nervous System', *Archives of Neurology*, 30 (1974), 36-38.

'Retroviruses without onc genes', Duesberg stated, are 'the most common and benign passenger viruses of healthy animals and humans.'<sup>40</sup> He argued that HIV is likely such a passenger virus with no significant pathological effect.

Duesberg elaborated his arguments in a second paper published in the *Proceedings of the National Academy of Sciences, U.S.A.*<sup>41</sup> A major element in his objection to HIV as the cause of AIDS is that the virus, in his judgment, fails to satisfy Koch's postulates. In both the *Cancer Research* and the *PNAS* papers, he explicitly sets forth his understanding of Koch's postulates:

1. The microorganism must be found in all cases of the disease.
2. It must be isolated from the host and grown in pure culture.
3. It must reproduce the original disease when introduced into a susceptible host.
4. It must be found present in the experimental host so infected.<sup>42</sup>

This version of Koch's postulates is obviously based on Koch's later work. In Duesberg's restatement of Koch's postulates, points 2, 3, and 4 all relate to the sufficiency argument: the organism must be cultured and demonstrated to induce the disease – that is, with the organism, there is disease. Because hosts can react differently to contact with pathogens, this line of argument is much more difficult to prove in the case of a new organism than is the necessity argument, stated in point 1: without the organism there is no disease.

Duesberg, it should be noted, cited Alfred Evans's 1976 review of methods of scientific proof, 'Causation and Disease', in the notes to his *PNAS* paper, indicating that he was aware of varied approaches to demonstrating causality.<sup>43</sup> Furthermore, he referred to and cited Robert Huebner's guidelines to demonstrating etiology of viruses, which Evans discussed, but he ignored Thomas Rivers's modification of Koch's postulates, also discussed by Evans.<sup>44</sup> One possible reason

<sup>40</sup> Peter H. Duesberg, 'Retroviruses as Carcinogens and Pathogens: Expectations and Reality', *Cancer Research*, 47 (1987), 1199-1220; quotation from p. 1200.

<sup>41</sup> Peter H. Duesberg, 'Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome: Correlation but not Causation', *Proceedings of the National Academy of Sciences, U.S.A.* 86 (1989), 755-64.

<sup>42</sup> Duesberg, 'Retroviruses as Carcinogens and Pathogens: Expectations and Reality', p. 1199, n. 3. In his paper, 'Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome: Correlation but not Causation', Duesberg utilizes a slightly different wording: 'The first of these postulates states that "the parasite must be present in every single case of the disease, under conditions that can account for the pathological lesions and the clinical course of the disease". ...Koch further postulated that it must be possible to isolate and propagate the etiological agent from all cases of the disease. ...Koch's third postulate calls for inducing the disease by experimental infection of a suitable host with pure pathogen'. See pp. 756-57.

<sup>43</sup> Duesberg, 'Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome: Correlation but not Causation', p. 763, n. 67.

<sup>44</sup> *Ibid.*, p. 763, n. 68.

for this is that Rivers's first postulate, 'A specific virus must be found associated with a disease with a degree of regularity', would weaken Duesberg's argument that HIV has not been found in every case of AIDS. In short, although Duesberg acquainted himself with Evans's discussion of the changing nature of Koch's postulates, he chose to frame his discussion rigidly within the limits of the classical version.

In 1988 *Science* magazine published a Policy Forum in which Duesberg and his critics summarized their arguments.<sup>45</sup> Those scientists supporting HIV as the cause of AIDS were William Blattner and Robert Gallo of the U.S. National Cancer Institute and Howard Temin of the University of Wisconsin, Madison – all three distinguished investigators. They, too, cited Alfred Evans's paper, using it to support their statement that Koch's postulates provide 'a useful historical reference point, but were not regarded as rigid criteria by Koch himself and should not be so regarded today'.<sup>46</sup>

Duesberg argued that HIV is in violation of Koch's first postulate because it is not possible to detect free virus, provirus, or viral RNA in all cases of AIDS. HIV also failed to meet Koch's second postulate, he stated, because it cannot be isolated from 20 to 50% of AIDS cases. Blattner and colleagues responded to these two arguments together. They pointed out that both detection and isolation of the virus were hampered by the limitations of existing laboratory methods and that increasingly sensitive tests show HIV infection 'in essentially all AIDS patients'.

In this exchange, both Duesberg and Blattner and colleagues have transformed Koch's second postulate – the isolation of the organism – into a restatement of the first postulate – the organism must be present in all cases of the disease. In earlier debates over etiology, the isolation of the organism in pure culture was not required in every case; it was instead linked with efforts to demonstrate that the organism was sufficient to cause disease in experimental animals. Understanding that both of these statements reflect the necessity argument, we must ask whether Duesberg is being too rigid – this is where Rivers's first postulate would have hurt his argument – in requiring that HIV be identified in *all* cases before accepting the agent as the cause of the disease. In contrast, we should also ask if Blattner and colleagues are

<sup>45</sup> Series of short papers in Policy Forum, *Science*, 421 (1988), 514-517. Specific titles of these papers: Peter Duesberg, 'HIV is Not the Cause of AIDS'; 'Blattner and Colleagues Respond to Duesberg'; W. Blattner, R. C. Gallo, and H. M. Temin, 'HIV Causes AIDS'; 'Duesberg's Response to Blattner and Colleagues'.

<sup>46</sup> Blattner and Colleagues Respond to Duesberg, p. 514; p. 517, n. 1.

too quickly discounting AIDS deaths in which HIV had not been found by prevailing methods.

Duesberg also contended that HIV is in violation of Koch's third postulate because 'pure HIV does not reproduce AIDS when inoculated into chimpanzees or accidentally into healthy humans.' Blattner and colleagues easily rebutted the chimpanzee portion of this argument, noting that 'most viruses are species-specific in host range and in capacity to produce disease'. They contended, furthermore, that HIV 'does indeed cause AIDS when inoculated into humans' who have no underlying medical condition that might also account for the resulting immunodeficiency. 'Accidental needlestick injuries with HIV-contaminated needles', they stated, 'have resulted in HIV seroconversion and then clinical AIDS'. This is true, but Duesberg's assertion that not every needlestick has resulted in seroconversion is also true. Since it is well known that host defense mechanisms vary, and since no disease organism produces frank infection in every contact with a host, the evidence from accidental needlesticks supports Blattner and colleagues' argument more strongly than it does Duesberg's.

In addition to the arguments based on Koch's postulates, Duesberg presented additional objections to HIV, most of which were easily refuted by Blattner, Gallo, and Temin. Utilizing the immunological model that proved useful in studies of hepatitis and infectious mononucleosis, Duesberg stated that it is paradoxical for HIV to cause AIDS only after the onset of antiviral immunity, arguing that all other viruses are most pathogenic before immunity is detected. He also asserted that the long incubation period, up to eight years or more, is bizarre for a virus that replicates in one or two days. In this argument, Duesberg appears to be requiring that HIV and other new retroviruses follow a specifically defined, disease-inducing process. Blattner and colleagues countered with the observation that many viruses are pathogenic after immunity appears and have long and variable latent periods. Research on unconventional slow viruses has also demonstrated long latency periods and viral activity without any detectable immune response.

Duesberg's observation that retroviruses typically promote cell growth rather than kill cells is similarly weak. Several retroviruses, such as Rous-associated virus-2, spleen necrosis virus, and visna virus all kill infected cells in culture and establish a chronic stage of infection in which surviving infected cells continue to divide. Weakest of all, perhaps, is Duesberg's argument that the epidemiology of AIDS indicates that the virus has a country-specific host range and a risk group-specific pathology. As Blattner and colleagues pointed out, the



epidemiology simply reflects the spread of the virus, which has depended on social and environmental factors in the different countries where it has been identified.

Duesberg put forth two final arguments that have intrinsic merit but that may not be relevant to the question of causation. He contended that the retrovirus 'is not biochemically active' in the disease, infecting only a small number of the T cells it is supposed to destroy. Blattner, Gallo, and Temin observed that the virus also infects macrophage and monocyte cells as well as T cells, and they argued that complete knowledge of the pathogenesis of the disease is not necessary to establish etiology. Second, Duesberg contended that it is highly improbable that the two viruses linked to AIDS, HIV-1 and HIV-2, could have evolved within the short time span that HIV has supposedly been in existence. Blattner and colleagues refuted this argument with the observation that although the question of the origin of these viruses is interesting, it has no bearing on whether HIV causes AIDS.

Blattner, Gallo, and Temin bolstered their case for HIV as the cause of AIDS with epidemiological evidence. Relying on serological testing methods, they noted that in every group studied prospectively, positive seroconversion was followed by progressive immunodeficiency and clinical AIDS 'in a predictable sequence'. They also cited cases of AIDS following blood transfusions and cases in infants of mothers with AIDS, both of which have been linked to HIV infection. Finally, they argued that interruption of HIV transmission by screening donated blood for HIV antibodies halted the further incidence of AIDS in blood transfusion recipients. 'Scientists conclude,' they state, 'that a virus causes a disease if the virus is consistently associated with the disease and if disruption of transmission of the virus prevents occurrence of the disease'.

This argument strongly resembles Koch's argument about the relation of the comma bacillus to cholera. It is based primarily on the necessity argument: without HIV there is no AIDS. Yet it also includes two weak sufficiency arguments: in most cases, the presence of HIV is followed by the onset of AIDS, and, in most cases of AIDS, HIV has been identified. William Blattner strongly reiterated his conviction about this in an interview. When asked what convinced him that HIV caused AIDS, he replied:

People who had intact T-cell subsets were not antibody positive; people who had severely depressed T-cell subsets had antibodies. People who had a lifestyle that put them at greatest risk for infection were infected; people who engaged in safe sex and did not abuse drugs were not as likely to be infected. Of people for

whom we had serological samples, those who sero-converted developed AIDS. People who did not sero-convert did not develop AIDS.<sup>47</sup>

Duesberg contended that epidemiological and serological evidence was not enough. Although he mentioned several specific objections, both his rebuttal to Blattner and colleagues and his own major argument hung on the possibility that HIV could be a nontoxic passenger virus and that some other pathogen or factor will be found to be the real cause of AIDS. Thus we return to Duesberg's demands that HIV be demonstrated sufficient to cause the disease. The rigid form in which Duesberg cast this requirement is, as yet, impossible to satisfy. Some people have not sero-converted when exposed, and some seropositive persons have not yet developed AIDS, even though the evidence is strong that they will.

Most scientists have dismissed out-of-hand Duesberg's objection to HIV as the cause of AIDS, yet his call for a higher order of proof than has yet been attained is not without merit. AIDS is a new disease, and we should not forget that Nature has surprised us in the past as we searched to understand disease etiology. Indeed, new information about mycoplasmas as a possible cofactor in AIDS has recently stimulated a new line of investigation. Mycoplasmas are bacteria that do not form a cell wall. Lacking the cell-wall proteins that elicit an immune response, these organisms can more easily 'hide' from the body's defenses.<sup>48</sup>

In 1989 Shyh-Ching Lo of the U.S. Armed Forces Institute of Pathology and his collaborators published information about a pathogenic mycoplasma, which results in wasting syndromes similar to AIDS in both humans and non-human primates.<sup>49</sup> Luc Montagnier of the Pasteur Institute in Paris and his associates also reported that tetracycline analogs can protect cells from HIV-induced cell lysis. This observation led them to suggest that a mycoplasma may be involved,

<sup>47</sup> William A. Blattner, interview by Victoria A. Harden and Dennis Rodrigues, March 2, 1990, National Cancer Institute, Bethesda, Maryland; copy in NIH Historical Office files.

<sup>48</sup> 'Mycoplasmas', in F. J. Tortora, B. R. Funke, and C. L. Case, *Microbiology: An Introduction*, 2nd ed., (Menlo Park, Calif.: Benjamin/Cummings, 1986), p. 289.

<sup>49</sup> Shyh-Ching Lo, James Wai-Kuo Shih, Perry B. Newton, III, Dennis M. Wong, Michael M. Hayes, Janet R. Benish, Douglas J. Wear, and Richard Yuan-Hu Wang, 'Virus-like Infectious Agent (VLIA) is a Novel Pathogenic Mycoplasma: *Mycoplasma incognitus*', *American Journal of Tropical Medicine and Hygiene*, 41 (1989), 586-600; S. C. Lo, M. S. Dawson, D. M. Wong, P. B. Newton, III, M. A. Sonoda, W. F. Engler, R. Y. Wang, J. W. Shih, H. J. Alter, and D. J. Wear, 'Identification of *Mycoplasma incognitus* Infection in Patients with AIDS: an Immunohistochemical, *in situ* Hybridization and Ultrastructural Study', *ibid.*, 601-16. See also C. Saillard, P. Carle, J. M. Bové, C. Bébèar, S. C. Lo, J. W. Shih, R. Y. Wang, D. L. Rose, and J. G. Tully, 'Genetic and Serologic Relatedness between *Mycoplasma fermentans* Strains and a Mycoplasma Recently Identified in Tissues of AIDS and non-AIDS Patients', *Research in Virology*, 141 (1990), 385-95.

acting synergistically with HIV in the production of pathogenicity.<sup>30</sup> Thus far this research has produced no conclusions about the role of mycoplasmas in AIDS. Many questions will need to be answered before any definitive link can be asserted.<sup>31</sup>

The discovery of mycoplasmas in connection with AIDS does not necessarily support Duesberg's skepticism about HIV. Their advent, however, underscores a key point, which, I believe, has been evident in the historical experience with Koch's postulates. Establishing beyond cavil the etiology of a disease requires time, experience, new scientific data, and meticulous technique. Although the power of laboratory methods may enhance the types of studies that can be done, the successful investigator, like Robert Koch himself, must possess those priceless attributes that Thomas Rivers advocated: 'common sense, proper training and sound reasoning'. The revision of Koch's postulates seems destined to continue. What changes may be required in research on the etiology of AIDS remain to be seen.

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<sup>30</sup> M. Lemaître, D. Guétard, Y. Hémin, L. Montagnier, and A. Aerial, 'Protective Activity of Tetracycline Analogs against the Cytopathic Effect of the Human Immunodeficiency Virus in CEM Cells', *Research in Virology*, 141 (1990), 5-16.

<sup>31</sup> Additional Articles on the Role of Mycoplasmas in AIDS Include D. Concar, 'A Second Agent?' *Nature*, 345 (1990), 753; 'Mycoplasmas in the AIDS Spotlight', *Science*, 248 (1990), 682-83; C. Leach, R. Detels, J. Giorgi, P. English, and J. Cherry, 'Relationship of other Infections to Progression of Disease in HIV Infected Men', International Conference on AIDS, 1989 5: 689 (abstract no. C. 787); F. A. Bauer, D. J. Wear, P. Angritt, and S. C. Lo, 'Mycoplasma *incognitus* Infection in Kidneys of AIDS Patients with AIDS-Associated Nephropathy', International Conference on AIDS, 1990 6 (1): 255 (abstract no. Th.B. 535); Lo. Shyh-Ching, D. J. Wear, and M. M. Hayes, 'In Vitro Antimicrobial Susceptibility Testing for the Newly Identified Pathogenic Human Mycoplasma-M. *incognitus*', International Conference on AIDS, 1990 6 (1): 256 (abstract no. Th.B. 536); I. H. Chowdhury, T. Munakata, Y. Koyanagi, S. Kobayashi, S. Arai, and N. Yamamoto, 'Mycoplasma can Enhance HIV Replication *in vitro*: a Possible Cofactor Responsible for the Progression of AIDS', *Biochemical and Biophysical Research Communications*, 170 (1990), 1365-70; M. B. Vasudevachari, T. C. Mast, and N. P. Salzman, 'Suppression of HIV-1 Reverse Transcriptase Activity by Mycoplasma Contamination of Cell Cultures', *AIDS Research and Human Retroviruses*, 6 (1990), 411-16; G. T. Spear, A. L. Landay, B. L. Sullivan, B. Dittel, T. F. Lint, 'Activation of Complement on the Surface of Cells Infected by Human Immunodeficiency Virus', *Journal of Immunology*, 144 (1990), 1490-96.