

Causation and Disease: The Henle-Koch Postulates Revisited¹

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The Henle-Koch postulates are reviewed in terms of their full validity in Koch's day and in light of subsequent developments. The changing guidelines developed for viral diseases, for viruses in relation to cancer and to chronic central nervous system infection, and for causative agents in chronic diseases are discussed chronologically. A set of guidelines for both acute infectious and chronic diseases is presented. The need for recognizing the role of the host and the spectrum of host responses, for sound biologic sense in evaluating causal roles of agents in disease, and for flexibility in adapting our guidelines to new knowledge are emphasized.

For nearly 100 years the Henle-Koch postulates have been used as a reference point in evaluating the causal relationship of a new infectious agent to a clinical disease with which it is associated. The postulates have also served as a point of departure for many concepts of causation of chronic diseases. This paper will review chronologically the concepts as they were first presented and their evolution and shortcomings in the elements of causation attending viral infections, chronic disease, and cancer.

In 1840, Jakob Henle enunciated the concepts of causation in a book which has been translated into English by Dr. George Rosen (1). Henle was then 31 years old and just starting his duties as professor of anatomy in Zürich (Fig. 1).² These concepts were further developed by Henle's pupil, Robert Koch (Fig. 2) and presented as part of lectures in 1884 and 1890 (2, 3). The essence of the postulates is included in his 1890 presentation before the International Congress in Berlin and is reproduced below in the original German:

Wenn es sich nun aber nachweisen liess: erstens, dass der Parasit in jedem einzelnen Falle der betreffenden Krankheit anzutreffen ist, und zwar unter Verhältnissen, welche den pathologischen Veränderungen und dem klinischen Verlauf der Krankheit entsprechen: zweitens, dass er bei keiner anderen Krankheit als zufälliger und nicht pathogener Schmarotzer vorkommt; und drittens, dass er, von dem Körper vollkommen isolirt und in Reinculturen hinreichend oft umgezüchtet, im Stande ist, von Neuem die Krankheit zu erzeugen; dann konnte er nicht mehr zufälliges Accidens der Krankheit sein, sondern liess sich in diesem Falle kein anderes Verhältnis mehr zwischen Parasit und Krankheit denken, als dass der Parasit die Ursache der Krankheit ist.

Translated into English, the three basic concepts can be summarized as shown in Table 1. He concluded that, if all these conditions could be satisfied, then the "occur-

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²Henle was born in Fürth, Germany, later the birthplace of Henry Kissinger.



FIG. 1. Jakob Henle, 1809–1885 (from “Jakob Henles Zürcher Jahre, 1840–1844,” by Guido Gozzi, Juris Druck and Verlag, 1974).



FIG. 2. Robert Koch, 1843–1910 (from “Robert Koch,” by Richard Bochalli, Wissenschaftliche Verlagsgesellschaft m.b.H., Stuttgart, 1954).

TABLE 1
Henle-Koch Postulates^a

1. The parasite occurs in every case of the disease in question and under circumstances which can account for the pathological changes and clinical course of the disease.
2. It occurs in no other disease as a fortuitous and nonpathogenic parasite.
3. After being fully isolated from the body and repeatedly grown in pure culture, it can induce the disease anew.

^aBased on Rivers' translation (1937).

rence of the parasite in the disease can no longer be accidental, but in this case no other relation between it and the disease except that the parasite is the cause of the disease can be considered." At the time of presentation Koch felt that certain human and animal agents fully fulfilled these criteria. These included the organisms causing anthrax, tuberculosis, erysipelas, tetanus, and many animal diseases; in particular, almost all diseases which are infectious for animals.

At the same time there were a number of infectious agents which did not meet all the criteria and yet which Koch felt were strongly implicated in the causation of the disease. These included the bacteria isolated from typhoid fever, diphtheria, leprosy, relapsing fever, and Asiatic cholera. The major problem of fulfillment was the inability "to produce the disease anew" in an experimental host. Koch felt keenly that cholera was due to the *Vibrio* because he himself had first discovered the organism and identified its constant relationship with the disease in an epidemic which he studied in India (4). Yet there was no experimental animal in which the disease could be reproduced. As a matter of fact Koch's contemporary, the great hygienist and chemist, Prof. Max Pettenkofer of Munich, felt that the disease was not directly reproduced by the organism found in the stool, but required the acquisition of some factor of virulence through residence in the soil (the so-called Bodentheorie). To prove this, Pettenkofer carried out his "experimentum crucium" in 1880 in which he swallowed 1.0 cm³ of a freshly grown broth culture from a case of cholera and did not develop the disease (5), even though he excreted large amounts of cholera *Vibrio*. Some of Pettenkofer's pupils did not fare quite as well and developed mild diarrhea when they later repeated the master's experiment. Pettenkofer *did* believe that the organism caused the disease *after* it acquired virulence in the soil. Despite brilliant epidemiological proof of transmission by drinking water by John Snow of England (6), Pettenkofer could neither reproduce these epidemiological observations in his studies of cholera epidemics in Germany nor accept them on their own merits.

Another problem limiting the application of the Henle-Koch postulates was the inability to grow many presumed human pathogens in the laboratory. This applied to many parasitic diseases, such as malaria, as well as to leprosy. Thus, even at the time they were presented, the Henle-Koch postulates were never recommended as rigid criteria of causation and failed to apply to many diseases at the time when a causal relationship seemed almost unequivocal; Koch himself felt that fulfillment of the first two postulates might be sufficient evidence of causality.

The second criterion, that the organism "occurs in no other disease as a fortuitous and non-pathogenic parasite," became difficult to fulfill after it was found that an organism could also occur during the following *asymptomatic* infections, and sometimes persisted for months and even years.

For example, during the prolonged asymptomatic or chronic carrier states of

³Now designated as HBAG.



FIG. 3. Thomas Milton Rivers, 1888–1962 (from “Perspectives in Virology III,” Hoeber Medical Division, Harper and Row, New York, 1963).

organism “A,” a disease due to organism “B” might intervene, thus making organism “A” a fortuitous and nonpathogenic one in the presence of an unrelated disease. While suggested by Pettenkofer as early as 1855, the actual existence of such carriers was delayed until 1893 when they were recognized by Koch himself in his studies of cholera in 1893 (4). Their importance in transmission of illness became abundantly clear in the work of Park and Beebe with diphtheria in 1894 (7), of Koch with typhoid fever in 1902 (8), and of Chapin in 1906 in the control of communicable diseases (8a).

VIRUS DISEASES

The discovery of viruses, their growth in mice and in embryonated eggs, and their role in human disease circa 1930 brought new problems in causation and reemphasized the limitations of the original Henle–Koch postulates. Dr. Thomas Rivers (Fig. 3), the distinguished American virologist, discussed these issues in his Presidential Address before the American Society of Immunology in 1937 (9). He pointed out that blind adherence to Koch’s postulates might sometimes act as a hindrance rather than an aid. For example, the idea that an infectious malady can be caused only by the action of a single agent is incorrect, and, if Shope had adhered to this notion, he would never have discovered that swine influenza as it occurs in nature is caused by the combined or synergistic action of two agents, one a virus not cultivable on lifeless media, the other an ordinary hemophilic bacterium (10). The requirement that the infectious agent be grown in a pure state on lifeless media also hindered the establishment of the causal relation of viruses to disease because viruses require living tissues for propagation, i.e., chick embryo or tissue culture. Thus, Rivers felt that “Koch’s postulates have not been fulfilled in viral diseases.” In their

TABLE 2
Rivers' Conditions for Establishing Specific Relation of a Virus to a Disease^a

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1. A specific virus must be found associated with a disease with a degree of regularity.
 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.
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^aFrom Rivers (9).

place he suggested two conditions to be met before the specific relation of a virus to disease is established (Table 2). In these criteria Rivers felt, first, that it is not obligatory to demonstrate the presence of virus in every case of disease produced by it; second, that asymptomatic virus carriers exist; and third, that it is not essential that a virus be grown on lifeless media or in modified tissue cultures. In illness, the specific causative virus should always be found at the proper time in specific lesions. In establishing proof of causation animals should become sick or die when inoculated with material from such tissues shown to be free of ordinary microbes or rickettsiae and it should be possible to transmit the disease serially. Rivers warned that one must be wary in doing this experiment as experimental animals are subject to their own viral diseases and such an agent might be picked up during passage; the occurrence of healthy viral carrier states in experimental animals may also lead to picking up the wrong agent in passage. External infection with other viruses being worked with in the laboratory may also occur.

In addition to recognition and isolation of the virus, Rivers also emphasized that "information concerning the presence of antibodies against the agent and the time of their appearance in the serum of patients is equally important as evidence of etiological significance of the virus" (9). He pointed out that "if a virus is the actual cause of a disease, immune substances are virtually absent from the patient's serum at the onset of illness and make their appearance during the period of recovery." He also recognized that recovery sometimes occurs without the development of antibodies, and occasionally a person possessing antibodies against a virus succumbs to a disease caused by it. These immunological aspects of causation suggested by Rivers are important to emphasize because of later problems in viral disease in which the agent could not be isolated or transmitted to laboratory animals and where proof of causation rested solely on these immunologic criteria.

THE EPIDEMIOLOGIC CONCEPT

While the Henle-Koch postulates involved the bacterial agent in causation and Rivers added elements of virological and immunological proof, it was Dr. Robert J. Huebner (Fig. 4) who emphasized another ingredient, the need for epidemiological data in establishing causation in viral diseases. In a 1957 paper entitled "The Virologist's Dilemma" he discussed the criteria for etiologic association of prevalent viruses with prevalent diseases (11). In his view the mere isolation of a viral agent having a temporal relation to the disease process, while necessary and of importance, provided a very low order of evidence for the purpose of proving causality. The virologist's dilemma was that improved technology had reduced "the isolation of new human and animal viruses from a technological feat of high order to an almost exasperatingly commonplace occurrence." At least 50 new viruses of man had been discovered and new and untyped agents by the hundreds were accumulating in freezers in virus laboratories far more rapidly than they could be characterized, classified, or associated in a meaningful way with the causation of specific diseases. A number of viruses such as those in the coxsackie, ECHO, and adenovirus groups could not only



FIG. 4. Robert J. Huebner, 1914– , Chief, Laboratory of RNA Tumor Viruses, National Cancer Institute, National Institutes of Health.

cause illness but also occurred commonly in apparently healthy persons, a viral flora. Indeed, in one nursery studied by Huebner all 43 infants were completely healthy in September 1955 yet all were carrying an ECHO-like virus which was isolated on 90 occasions in that single month. In subsequent months the nursery returned to a more “normal” state with high weekly rates of febrile illness. This same high frequency of

TABLE 3

Huebner’s Prescription for the Virologist’s Dilemma: Conditions Necessary for Establishing a Virus as Cause of a Specific Human Disease^a

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1. *Virus must be a “real” entity:* A new virus must be well established by passage in the laboratory in animal or tissue cultures.
 2. *Origin of Virus:* The virus must be repeatedly isolated from human specimens and shown not to be a viral contaminant of the experimental animals, cells, or media employed to grow it.
 3. *Antibody response:* An increase in neutralizing or other serologically demonstrable antibodies should regularly result from active infection..
 4. *Characterization and comparison with known agents:* A new virus should be fully characterized and compared with other agents including host and host-cell ranges, pathologic lesions, types of cytopathogenic effects, size, susceptibility to physical agents, etc.
 5. *Constant association with specific illness:* The virus must be constantly associated with any well-defined clinical entity and isolated from diseased tissue, if available.
 6. *Studies with human volunteers:* Human beings inoculated with a newly recognized agent in “double blind” studies should reproduce the clinical syndrome.
 7. *Epidemiologic studies:* Both “cross-sectional” and “longitudinal” studies of community or institutional groups to identify patterns of infection and disease.
 8. *Prevention by a specific vaccine:* One of the best ways to establish an agent as the cause.
 9. *Financial support:* A consideration so absolutely necessary that it deserves to be called a postulate.
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^aFrom Huebner (11).

minor illnesses and a plethora of prevalent viruses have been found in family settings in New Orleans, New York, and Seattle by Fox in a series of excellent studies carried out in these cities over several years under the heading "The Virus Watch Program" (12-14). These observations of wide dissemination, high prevalence, numerous immunologic types, and the frequent isolation of viruses from persons who were healthy or had only mild illnesses led Huebner to make a list of suggestions for establishing causality which incorporated elements of both Koch's and Rivers' postulates. He suggested these might be called a "Bill of Rights for Prevalent Viruses" to bring a legal concept to an area of scientific research and to comprise a guarantee against "the imputation of guilt by simple association." These conditions are outlined in Table 3 along with his comments. He also emphasized the need for specific hyperimmune sera against newly recognized viruses as a means of identifying and classifying them. Huebner cautioned that his suggestions should not be regarded as "postulates," or even as advice to forlorn virologists, but merely as useful guidelines.

As the causative agent of many specific clinical entities became established, it became apparent about 1960 that a number of common illnesses or syndromes existed in which several agents could produce the same clinical picture (15). It was also recognized that despite intensive investigation *no* causative agent could be identified in 25 to 50% of common acute respiratory syndromes, in 75% or so of acute febrile clinical syndromes involving the central nervous system, or in most cases of common acute gastroenteritis. This led to the formulation of a group of "Five Realities" for acute respiratory disease, shown in Table 4 (16). These concepts greatly complicated

TABLE 4
The Five Realities of Acute Respiratory Disease^a

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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.
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^aFrom Evans (16).

establishing proof of causation; it became possible to state only that a given virus or other infectious agent was *one* of the causes of a given respiratory, gastrointestinal, or central nervous system syndrome and that its causal involvement might be apparent only in certain years, or months of a year, or in certain age groups, or in military personnel and not in civilian populations, etc. For example, rhinoviruses are associated with only 20-25% of the common cold syndrome and are active mostly in the fall. Respiratory syncytial virus is the major cause of severe respiratory disease in infants but produces mild or even asymptomatic infections in older children and adults; adenoviruses types 4 and 7 are very important causes of acute respiratory disease in military recruits but of little consequence in young adults in civilian settings.

Thus the establishment of a causal relationship of a given virus with a given syndrome became possible only under a set of special circumstances. The Henle-Koch postulates were concerned primarily with the nature of the *agent* in establishing causality; to these was added the need to consider the *circumstances* under which infection occurred; and finally the influence of the *host* in determining whether clinical disease develops was recognized.

AGENTS IN SEARCH OF DISEASE

While the development and utilization of chick embryo, suckling mice, and tissue culture techniques for isolation of viruses led to the discovery of a multitude of agents for which causal relationships to disease had to be worked out, technological developments in the late 1960's and early 1970's created a new problem. These techniques permitted identification of a new agent by the electron microscope or by serological techniques even though the agent itself could not be isolated and/or propagated in the laboratory. This made it impossible to apply Henle-Koch's, Rivers', or Huebner's guidelines of causation. In some instances the "agent" identified was found in healthy persons or in persons with a chronic disease and the relationship to an acute infectious disease, if any, was not immediately apparent. The two best examples of this situation were the discovery of Australia antigen in 1965 by Blumberg (17) in the serum of a healthy aboriginal native of Australia through immunodiffusion techniques and the demonstration of herpeslike particles under the electron microscope by Epstein, Barr, and Achong in 1964 (18) in cells cultured from a biopsy of an African lymphatic tumor described by Burkitt (19). In neither instance could the "agent" be isolated or grown in pure culture in the laboratory. The serologic technique for identifying Australia antigen in the serum of human populations was simple enough to permit an epidemiologic search for possible diseases to which it might be related. Similarly, in 1966 the Henle's worked out an immunofluorescent antibody technique for the herpes virus, now called Epstein-Barr or EB virus which also could be applied as an epidemiologic tool (20). The search for unknown diseases associated with these two agents, if any, was the reverse of previous efforts to search for agents causing known diseases. Because they could not be grown in tissue culture the search for disease associations depended on sero-epidemiological techniques.

A protocol for this type of pursuit is outlined in Table 5. Following this approach an association of Australia antigen² with viral hepatitis (type B) was established (21). Further support for causation came from the longitudinal studies of Krugman and Giles at Willowbrook School and their deliberate exposure experiments (22, 23). Subtypes of the antigen are now recognized (24-26) and methods of antibody assay have been developed (24). These permit most sophisticated techniques for evaluating causality.

In 1968 the grandson of Jakob Henle, Dr. Werner Henle of Philadelphia (Fig. 5), made a claim of causality that met none of the famous Henle-Koch postulates (1). With his wife, Gertrude, and a young German physician, Volker Diehl, they claimed that Epstein-Barr virus (EBV), or a close relative of it, was the cause of infectious

TABLE 5
Protocol for Seroepidemiological Pursuit of Agents in Search of Disease

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1. Develop a serological technique which is simple, specific, and sensitive enough to be applied on a large scale to epidemiologic investigation.
 2. Determine the prevalence of the component in sera obtained from different geographic areas, age and sex groups, and socio-economic settings to learn its distribution in healthy population groups.
 3. Test acute and convalescent sera from different clinical syndromes, especially those of unknown cause in which there is some ground for suspecting a causal relationship.
 4. If an association with a disease is found:
 - a. Establish the specificity of the association and of the antibody produced.
 - b. Initiate prospective studies
 - i. to show that persons possessing antibody are immune, those lacking it are susceptible;
 - ii. to determine the incidence of infection and of disease in persons lacking antibody.
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FIG. 5. Werner Henle, 1910– , Professor of Virology and Pediatrics, School of Medicine, University of Pennsylvania, a grandson of Jakob Henle.

mononucleosis (27). The evidence presented was immunological in nature, rather than based on the demonstration or isolation of the causative agent from clinical specimens or the reproduction of the disease anew in an animal model. By sero-epidemiological techniques they found that antibody to EBV was worldwide in distribution and that the age distribution in American children paralleled that of antibodies to other common viruses such as measles, mumps, and poliomyelitis in the prevaccination era. No lead was uncovered by testing paired sera from pediatric patients with unidentified viral infections or sera obtained during prospective studies of viral infections (27). The key clue to the illness associated with this virus arose unexpectedly when their technician, E.H., developed infectious mononucleosis. Her plasma devoid of EB antibodies prior to illness contained antibody in a titer of 1:40 during acute illness; her leukocytes, which could not be grown in culture prior to illness, now grew well within 4 weeks, and 4 weeks later were shown to harbor EBV antigen. This observation led to a study in which all of 42 Yale students with acute infectious mononucleosis were found to have EBV antibody, whereas of 50 sera randomly selected from healthy Yale controls only 24% had antibody. Finally, they showed that EBV antibody was absent in preillness sera from 12 patients who later developed

infectious mononucleosis accompanied by the appearance of antibody. These findings have now been amply confirmed (28, 29). Antibody to EBV is regularly absent prior to illness and regularly appears during clinical infectious mononucleosis; the presence of this antibody at the start of the prospective observation period is a reliable indicator of immunity to infectious mononucleosis, and its absence is a reliable indicator of susceptibility to EBV infection and to clinical infectious mononucleosis (29–33). The consistency and longitudinal nature of the epidemiological data established on immunological grounds alone that EBV was beyond reasonable doubt the cause of heterophile positive infectious mononucleosis (34). It was also shown that both EBV (30) and cytomegalovirus (34a) could cause a similar syndrome lacking heterophile antibody. Additional findings of EB viral excretion in the throat, the presence of EBV in lymphocytes cultured from the blood, and the reproduction of some features of the disease in experimental animals provided further proof of causation and is reviewed elsewhere (29, 34). However, current data are still inadequate to fulfill the Henle-Koch postulates since the agent is present in healthy carriers, has not been isolated in pure culture, and the disease has not been fully “reproduced anew” experimentally. These studies resulted in the development of a set of *immunological* criteria of causation as shown in Table 6. These criteria have been fulfilled

TABLE 6
Elements of Immunological Proof of Causation^a

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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.
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^a Derived from Evans (29).

for EBV as a cause of infectious mononucleosis and may also be applicable to other situations in which the causative agent cannot be grown in the laboratory nor a susceptible experimental animal found. A sixth and final criterion to be fulfilled after the agent has been isolated and grown in pure culture is that “production of antibody to the agent (immunization) should prevent the disease, or at least that portion of the syndrome attributable to that agent.”

CHRONIC VIRAL INFECTIONS OF THE CENTRAL NERVOUS SYSTEM (CNS)

The immunological criteria are not applicable to certain *unique* agents which are associated with kuru and Creutzfeld–Jakob disease. These conditions are characterized by progressive central nervous system involvement and death. In these patients no immune response is detectable, and the viruses cannot be isolated in the laboratory. The incubation period is very long and the agents extremely resistant to physical and chemical treatment. The criteria of causation depend on transmission of the disease to experimental animals. Dr. R. Johnson (Fig. 6) and C. J. Gibbs (35) have presented an excellent discussion of this problem and drawn up a set of criteria which is presented in Table 7. Scrapie disease of sheep and a transmissible encephalitis of mink are comparable infections of animals.

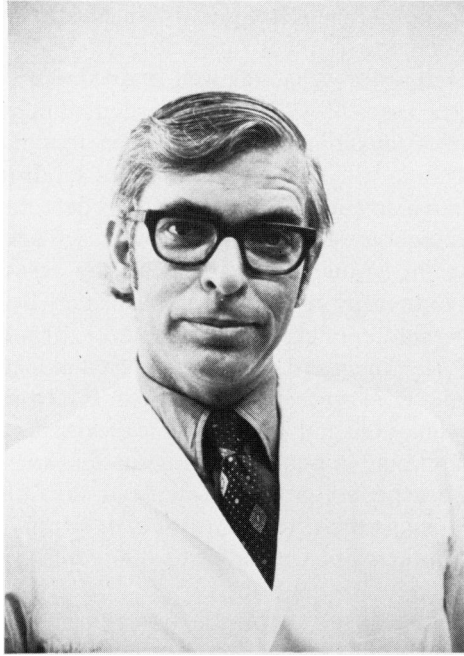


FIG. 6. Richard T. Johnson, 1931– , Professor of Neurology, School of Medicine, Johns Hopkins University.

There is also the problem of the causal relationship of *common* and persistent viruses to certain chronic central nervous system diseases because (i) primary infection often occurs early in life, is ubiquitous, and may not be attended by a recognizable clinical illness, and (ii) reactivation occurs years later, is rarely clinically expressed, and often is found under circumstances associated with a depression of cell-mediated immunity. The relation of measles virus to subacute sclerosing panencephalitis (SSPE) (37) and of papova virus to progressive multifocal leukoencephalopathy (PML) (38) are examples of this. Depression of the immune state both with regard to persistence of the agent and of its reactivation may be required. For the measles/SSPE relationship the causal proof rests on (a) the presence of measles antibody, including IgM, in serum and spinal fluid; (b) high serum antibody titers to the virus; (c) demonstration and/or isolation of the virus or of viral antigen in affected tissues; and (d) experimental production of a similar disease in hamsters (39). These do not fully fulfill preexisting concepts of causation and it is necessary to

TABLE 7
Johnson and Gibbs Criteria for Relating Slow Viral Infections and Chronic Neurological Disease^a

First, 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.

Second, 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.

Third, 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.

^aFrom Johnson and Gibbs (35).

exclude the presence of other antigens in brain tissue. Measles virus might simply be a passenger in brain tissue that is reactivated during some other illness. The syndrome might also be due to other viruses as well. Indeed, EBV has been found along with measles virus in three cases of SSPE under circumstances in which, had it been detected alone, suspicion of causation might have fallen upon it (40); rubella virus has also been found in association with a SSPE-like clinical syndrome (41). It is possible that the healthy brain, like many other tissues, contains detectable antigenic residues of many past systemic infections. If such viral residues are demonstrated in persons dying of a CNS disease they would be found guilty by association. Furthermore, under immunosuppressive therapy one or more viruses may be reawakened to active multiplication. Such reactivation might be asymptomatic or might produce a clinical disease like SSPE or PML. The increasing use of brain biopsy in clinical diagnosis, the means of identifying viral antigens by use of electron microscopy and by fluorescent antibody techniques, and the isolation of viruses by cocultivation with other tissues or in embryonic human brain-tissue cultures have permitted the recognition of several unsuspected viruses in nerve tissue. Just as the presence of viruses in other tissues such as the throat, intestine, or lymph tissues does not alone establish causation, so too their presence in central nervous system tissue must be interpreted with caution.

VIRUSES AND CANCER

The obstacles in establishing a possible etiological role of a virus in cancer include (i) the long "incubation" period between exposure to the suspected agent and the development of the disease; (ii) the relatively low incidence of most cancers which makes prospective studies almost impossible because of the large number of persons who must be kept under observation; (iii) the possibility that cancer may result as a consequence of a *reactivated viral* infection years later rather than as a direct consequence of a *primary* infection; (iv) the widespread and ubiquitous nature of the viruses under greatest suspicion of oncogenicity; (v) the probable role of infectious, environmental and/or genetic cofactors in producing cancer; (vi) the difficulty in fully reproducing the disease in animals, and (vii) the impossibility of human experimentation with potentially oncogenic agents.

The most likely candidates for human oncogenic viruses are two herpes viruses, EBV and herpes simplex virus type 2. In animals, evidence implicating the herpes group of viruses in the causation of tumors has been published for Marek's disease in chickens (42), Lucké adenocarcinoma in frogs (43), and herpes saimiri and herpes ateles virus in monkeys (44). The prevention of tumors in Marek's disease by the administration of a virus vaccine very early in life provides strong support favoring causation of this tumor by the virus (45). Similarly, evidence of vaccine-induced protection against herpes-associated tumors of monkeys has been recently reported (46).

In man, the relation of EBV to Burkitt lymphoma and to nasopharyngeal cancer based on serological and virological evidence is reviewed elsewhere (34, 47). The challenge of establishing immunological evidence of causation of EBV in Burkitt lymphoma with prospective studies as used for infectious mononucleosis (Table 7) was formidable because of the low incidence of the cancer, about 8-10/100,000 even in highly endemic tumor areas, the requirement for a large population group whose sera had to be tested, and the need for clinical surveillance over a long period of time in settings where medical and diagnostic facilities are inadequate. Despite these difficul-

TABLE 8
Sero-epidemiological Criteria Relating Viruses to Cancer

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1. The specific antibody must be present more often in sera of patients with the cancer than in healthy age/sex matched controls living in the same area.
 2. The antibody levels to the virus must be elevated over those found in healthy controls as shown by
 - a. a significantly higher geometric mean antibody titer;
 - b. a significantly higher frequency of elevated titers over a pre-set level than that in healthy controls.
 3. Antibody characteristics suggesting persisting or reactivated viruses, such as antibody to early antigen, IgM antibody, etc., should be present more commonly and at a higher level than in controls.
 4. The specificity of the antibody relationship should be established by showing
 - a. that the antibody is specific for that virus;
 - b. that antibodies to other viruses are not elevated under similar circumstances.
 5. Prospective sero-epidemiological studies should establish either
 - a. that antibody is absent *prior* to onset of disease, if the condition is a consequence of primary infection or
 - b. that high levels of antibody *precede* the disease if the condition is a consequence of reactivation.
 6. If the association between the virus and the cancer is seen under some circumstances but not under others, then the presence of a cofactor (or cofactors) should be sought (infection, drug, genetic predisposition, socio-economic or behavioral factor).
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ties a massive prospective serological study of about 45,000 children in the West Nile District of Africa was launched in 1971 (48). At present (December 1975) eight cases of Burkitt lymphoma have occurred. EBV antibody was already present in the sera collected at the start of the observation period from all these cases prior to the appearance of tumor (49). This suggests that the tumor is not an immediate consequence of a primary EBV infection and, if related, would represent a persistent or reactivated infection. This also creates difficulty in establishing causality since the presence or absence of the antibody alone cannot be used as the epidemiologic marker. It is the *height* and *characteristics* of the antibody in sera from patients with Burkitt lymphoma that must be compared with control groups. This type of serological evidence is summarized in Table 8. Of itself it can only be regarded as establishing an association and not proof of causation. An important element of immunological proof, and one difficult to carry out logistically, is to determine prospectively if the risk of Burkitt lymphoma (BL) and of nasopharyngeal cancer (NPC) (50) is significantly higher in persons with high antibody levels *prior* to tumor development as opposed to the risk of tumor in persons with normal levels of EBV antibody or no antibody at all. Preliminary evidence from the massive prospective study in the West Nile area of Africa indicates that *high* EBV antibody levels were present in the *preillness* serum (49). The need to consider cofactors like malaria for BL and genetic susceptibility for NPC must also be kept in mind in assessing this risk. Virologically, some of the elements of causation are shown in Table 9, most of which have been fulfilled in Burkitt's lymphoma. The presence of the EBV genome in every African Burkitt lymphoma cell is strong circumstantial evidence of association fulfilling the first Henle-Koch postulates (Table 1); the tumor is monoclonal suggesting that EBV must have been present in the malignantly transformed cell at the start. The re-

TABLE 9
Association of Viruses and Cancer: Virologic Evidence

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1. Evidence for *viral multiplication* (i.e., viral excretion).
 2. *Presence of virus* and/or viral genome in affected tissues.
 3. Demonstration of the ability of the virus to induce malignant transformation of cells *in vitro*.
 4. *Reproduction* of disease in animal model with purified virus.
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production of a malignant lymphoma in cotton top marmosets (51) and in owl monkeys (52) with EBV-infected lymphocytes or semipurified virus moves toward fulfillment of postulate 3 of Henle-Koch. However, the reported findings suggestive of a second, RNA-type virus, in Burkitt lymphoma cells (53, 54), if confirmed, raises the question of separate vs combined causality (or neither). These issues and those relating to the presence of contaminating viruses from the animals themselves are those discussed by Rivers almost 30 years ago (9).

Another herpes virus has been incriminated in cervical cancer. The relationship between herpes simplex virus, type 2, and this cancer is based on serological and virological evidence similar to that outlined for EBV and Burkitt lymphoma (Tables 8 and 9) but at the moment the evidence of causation is far less convincing (in the opinion of this reviewer, at least). There are difficult technical problems (See the recent review of Klein, G., The Epstein-Barr virus and neoplasia. *New Engl. J. Med.* **293**, 1353-1357 (1975)). For example, the laboratory differentiation between the widely prevalent herpes type 1 antibody and the type 2 antibody incriminated in cervical cancer has not been unequivocally established by a simple and reproducible test. Different workers use different methods. There are cross reactions between the two antibodies and HSV type 2 almost never occurs in the absence of HSV type 1 since it is acquired later in life. Hope of a more specific antibody marker with a purified virus has been provided by Aurelian (55). Large-scale prospective studies to establish risk ratios are needed, and some are in progress. An experimental animal host is badly needed. Good reviews of the relationship of this virus to cervical cancer have recently appeared (56, 57). Just as an association has been found between EBV in African but not American Burkitt lymphoma, so geographic differences in HSV type 2 and cervical cancer also exist. It appears that there is a five- to tenfold higher relative risk of invasive cervical cancer in individuals with HSV 2 antibody as compared to those without antibody in Brussels, Copenhagen, and Atlanta (in Negroes) but a relatively low risk or no increased risk in Israel, Auckland, and Yugoslavia (57). Epidemiologically, the most striking association of cervical cancer is not with herpes virus per se but with sexual intercourse. The age of onset and number of sexually active years, the frequency of the act, and the multiplicity of sexual partners (56, 57) are all positively correlated with the risk of cervical cancer. A venereally transmitted oncogenic agent other than HSV 2 in some areas of the world (or even in the United States) has not been fully excluded; several agents may be able to induce cancer.

With both EBV and HSV 2 it is clear that the age, geographic location, and the behavioral, genetic, and immunologic characteristics of the infected host play a decisive role in the development of cancer with agents as prevalent and ubiquitous as the herpes viruses. To paraphrase the words of Cassius in Shakespeare's *Julius Caesar* (58): "The fault, dear Brutus, lies not in our viruses, but in ourselves, that we are cancerlings." Even in infectious diseases Stewart (59) has stressed the need for considering host factors such as susceptibility, genetic constitution, behavior, and socioeconomic determinants in relation to causation, just as Paul has done in the relation of the *Streptococcus* to rheumatic fever (59a).

CAUSATION AND CHRONIC DISEASES

The recognition of an association between smoking and lung cancer in 1950 (60) and later of diet (61) and cholesterol (62) in relation to coronary heart disease stimulated efforts to draw up criteria for procedural and inferential methods in establishing causation. In a 1958 conference on this subject Dr. Yerushalmy (Fig. 7) and

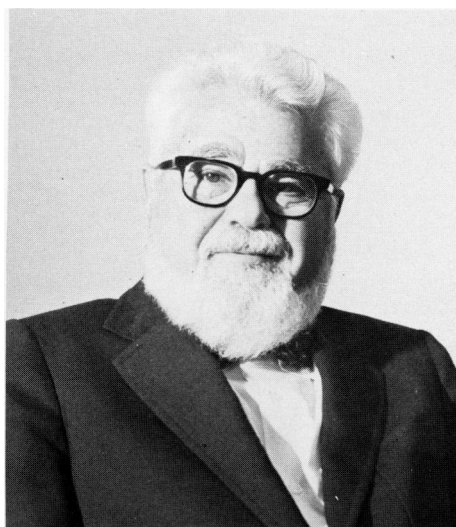


FIG. 7. Jacob Yerushalmy, 1904–1973, Professor of Biostatistics, University of California, Berkeley.

Palmer (63) developed a set of suggested criteria. First they reviewed Koch's postulates and stressed that in view of their possible application to chronic disease two essential types of evidence related to (i) the simultaneous presence of the organism and disease and their appearance in the correct sequence and (ii) the specificity of the effect of the organism on the development of the disease. In particular, the need was stressed to establish the *specificity* of an effect in studies of chronic diseases. The concept of multiple causation was also emphasized in chronic disease as opposed to acute infectious disease.

In applying the criteria three problems were mentioned: (i) the difficulty of measurement; (ii) the selection of controls against whom an increase in frequency may be gauged, and (iii) the question of the correct sequence of events. The equivalent in chronic disease for Koch's first postulate might take one of two forms which are presented as statements 1 and 2 of Table 10. The two statements were not to be regarded as independent. Retrospective studies are usually required to establish statement 1 and prospective studies to establish statement 2. It should not be necessary, in their view, to satisfy both statements although statement 1 does not in-

TABLE 10
Guideposts for Implication of a Characteristic as an Etiologic Factor in a Chronic Disease^a

1. The suspected characteristic must be found more frequently in persons with the disease in question than in persons without the disease, or
2. Persons possessing the characteristic must develop the disease more frequently than do persons not possessing the characteristic.
3. An observed association between a characteristic and a disease must be tested for validity by investigating the relationship between the characteristics and other diseases and, if possible, the relationship of similar or related characteristics to the disease in question. The suspected characteristic can be said to be specifically related to the disease in question when the results of such investigation indicate that similar relationships do not exist with a variety of characteristics and with many disease entities when such relationships are not predictable on physiologic, pathologic, experimental, or epidemiologic grounds. In general, the lower the frequency of these other associations, the higher is the specificity of the original observed association and the higher the validity of the causal inference.

^aFrom Yerushalmy and Palmer (63).

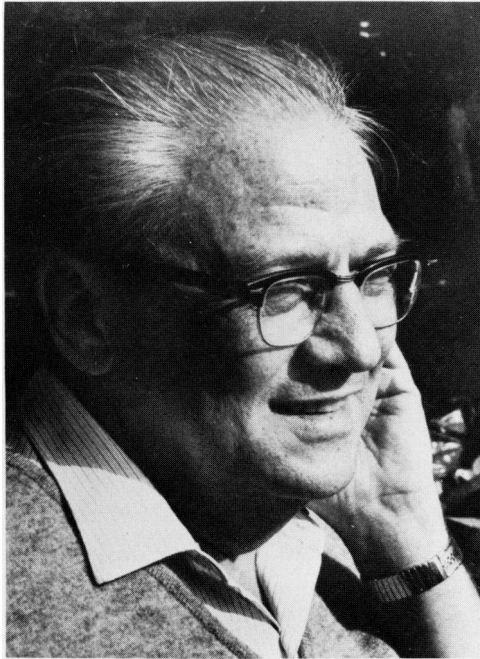


FIG. 8. Abraham M. Lilienfeld, 1920– , Professor of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University.

volve testing for the correct sequence. Yerushalmy and Palmer were most concerned with the selection of controls. For example, a comparison between lung cancer in smokers and in nonsmokers was not valid unless other characteristics were shown to be similar to the two groups because it might be some other characteristic of the smoker, other than smoking itself, that might be the real risk factor. For discussion purposes they presented a third statement relevant to this issue which is presented as item 3 in Table 10.

In a discussion of this early effort Lilienfeld (Fig. 8) pointed out that one might consider “vectors” in chronic diseases just as well as in infectious diseases; i.e., that cigarette smoke is the “vector” of lung cancer just as polluted water is the vector of typhoid fever and cholera (64). While such knowledge is important, the identification of the *specific* agent transmitted via the vector need not be necessary to develop practical methods of control. This elimination of exposure to polluted water can control typhoid fever and the elimination of exposure to cigarette smoke can reduce lung cancer without the need to identify the typhoid bacillus or the carcinogen in cigarette smoke. Certain additions and modifications to the Yerushalmy/Palmer concepts

TABLE 11
Added Criteria in Establishing Causation in Chronic Disease^a

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1. The incidence of the disease should increase in relation to the duration and intensity (dose) of the suspected factor.
 2. The distribution of the suspected factor should parallel that of the disease in all relevant aspects.
 3. A spectrum of illness should be related to exposure to the suspected factor.
 4. Reduction or removal of the factor should reduce or stop the disease.
 5. Human populations exposed to the factor in controlled studies should develop the disease more commonly than those not so exposed.
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^aBased on Lilienfeld (64–66).

were made by Lilienfeld in this (64) and other publications (65, 66; Table 11). The interplay of different factors in causation has been called "the web of causation" by MacMahon *et al.* in their text (67). The problem of causation has also been reviewed in a recent book by Susser (68) and in an article by Bollet (68a).

The problem of establishing objective criteria for causation was a direct challenge to the Surgeon General's Advisory Group on Smoking and Health in 1964 (69). They set up a series of attributes that related evidence of association between a suspected cause (smoking) and a disease (lung cancer) as summarized in Table 12.

TABLE 12
Elements of Causation in Chronic Disease^a

-
1. The consistency of the association.
 2. The strength of the association.
 3. The specificity of the association.
 4. The temporal relationship of the association.
 5. The coherence of the association.
-

^aFrom "Smoking and Health" (69).

DISCUSSION

There are a number of limitations that the investigator in acute infectious as well as in chronic diseases should recognize in pursuit of causality. The most important of these are: (i) The same *pathologic or clinical state* can be produced by different etiological agents. (ii) These causative agents may vary in different geographic areas, in different age groups, or with different patterns of host susceptibility. For chronic diseases the term "attributable risk" has been used to identify the proportion of a specific disease associated with a given cause (70, 71). Elimination of that cause should decrease the incidence of the disease by this amount provided the control measures are applied in exactly the same settings as those in which the attributable risk has been measured. For example, abstention from smoking should reduce lung cancer by 70%, other factors being constant, just as effective control of rhinoviruses would reduce the incidence of the common cold by 25%. (iii) Some diseases require the presence of two or more agents or cofactors acting together to produce the disease. (iv) A single agent may produce different clinical and pathological responses in different settings. (v) Any cause or set of causes usually produces a biologic gradient of response which may vary from no observable or even detectable reaction to mild clinical or pathologic changes, to classic and recognized disease. (vi) The nature and severity of the host response following exposure to an infectious or noninfectious agent varies with certain host characteristics, of which behavioral patterns, the socio-economic level, genetic constitution, age, immunological status, and exposure to other cofactors (infection, drugs, etc.) are the most important.

A causative agent of low pathogenicity or low potency may be able to induce clinical disease only in human hosts rendered more susceptible by exposure at a given age, or when resistance is impaired, or in the presence of a cofactor. When causal agents of this type are widespread (herpes viruses) or involve a very common substance (smoking, fat) it may be more efficient from the standpoint of prevention to identify the pertinent host factor and modify it rather than prevent exposure at all. In other instances control of a cofactor or of the vehicle of transmission may be the easiest route to prevention. On the other hand, there are microbial agents of high pathogenicity (rabies virus) or substances of high potency to which exposure is relatively uncommon and in which host susceptibility plays a minor role. In these settings the major effort should be to control exposure to the causal agent itself.

TABLE 13
Criteria for Causation: A Unified Concept

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1. *Prevalence* of the disease should be significantly higher in those exposed to the putative cause than in cases controls not so exposed.^a
 2. *Exposure* to the putative cause should be present more commonly in those with the disease than in controls without the disease when all risk factors are held constant.
 3. *Incidence* of the disease should be significantly higher in those exposed to the putative cause than in those not so exposed as shown in prospective studies.
 4. *Temporally*, the disease should *follow* exposure to the putative agent with a distribution of incubation periods on a bell shaped curve.
 5. A *spectrum* of host responses should follow exposure to the putative agent along a logical biologic gradient from mild to severe.
 6. A *measurable host response* following exposure to the putative cause should *regularly appear* in those lacking this before exposure (i.e., antibody, cancer cells) or should *increase* in magnitude if present before exposure; this pattern should not occur in persons so exposed.
 7. *Experimental reproduction* of the disease should occur in higher incidence in animals or man appropriately exposed to the putative cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the laboratory, or demonstrated in a controlled regulation of natural exposure.
 8. *Elimination or modification* of the putative cause or of the vector carrying it should decrease the incidence of the disease (control of polluted water or smoke or removal of the specific agent).
 9. *Prevention or modification* of the host's response on exposure to the putative cause should decrease or eliminate the disease (immunization, drug to lower cholesterol, specific lymphocyte transfer factor in cancer).
 10. The whole thing should make biologic and epidemiologic sense.
-

^aThe putative cause may exist in the external environment or in a defect in host response.

The common nature of these considerations of causation for both acute and chronic disease permits the development of a "unified concept" for consideration as criteria of causality (Table 13). The Henle-Koch postulates are a useful historical reference point but were not regarded as rigid criteria by Koch himself and should not be today. In view of later knowledge of carrier states and host responses, the postulates are simplistic and not in accord with today's facts. Insistence on their fulfillment before causality is accepted with a new agent in relation to a disease should be abandoned. If fulfillment of the postulates *can* be demonstrated, then a causal relation is probable. If not, then this does not eliminate the agent as the cause or one of the causes of the disease. Similarly, the criteria later evolved for viral diseases (9, 11), for immunologic proof (29), for slow infections of the central nervous system (35), and for chronic diseases (63, 64) must also be viewed in light of the technology available at the time of their formulation. The limitations of the methods of study will determine which, if any, of the published criteria will be useful for a given situation. New technology, better understanding of disease agents and of host responses, and the discovery of new "causes" will require changes in existing criteria. The ingenuity of the investigator will be taxed in this effort. As Rivers wisely noted in 1937 (9): "To obtain the best results, however, this ingenuity must be tempered by the priceless attributes of common sense, proper training, and sound reasoning."

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