

VIRUSES AND KOCH'S POSTULATES¹

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Diseases at one time were thought to be caused by wrath of the gods, configuration of stars or miasmas. After a real struggle that occurred not so many years ago, certain maladies were shown to be induced either by small animals or minute plants, *e.g.*, protozoa, fungi, bacteria and spirochetes. Indeed, the victory was so great that most workers in time began to consider that all infectious diseases, including those whose incitants had not been discovered, must be caused by agents similar to those already recognized. According to them, there could be no infections that were not caused by protozoa, fungi, bacteria or spirochetes, and to intimate that some infectious agents might be inanimate constituted heresy of the first order. Even at the present time, the cause of certain diseases is said by some individuals to be unknown or undiscovered, because no cultivable bacterium or visible protozoan parasite of etiological significance has been demonstrated in them. For instance, a few years ago Cowie made the statement in a scientific paper that the etiological agent of poliomyelitis is unknown, and in the recent book, *An American Doctor's Odyssey*, Heiser remarked that "the microbe which causes small-pox has never been discovered."

In spite of the general acceptance of the idea that all infectious diseases are caused by protozoa, fungi, bacteria or spirochetes, some workers have always contended that there might exist other infectious agents incapable of classification with those already known. Furthermore, very early in the bacteriological era a few discerning individuals appreciated the fact that there was no reason, except analogy, for assuming that all infectious agents must be living autonomous organisms. Through the activities

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of these investigators a group of disease-producing agents, known as viruses, has gradually become recognized. The exact nature of these agents is not known; some may be the midgets of the microbial universe, others may represent forms of life unfamiliar to us, while still others may be inanimate incitants of disease. Regardless of lack of complete knowledge of their nature, it is decidedly incorrect to say that these agents are unknown. The incitants of smallpox, vaccinia, poliomyelitis, yellow fever, fowl plague and tobacco mosaic are known; they can be recognized or identified in a variety of ways; they can be separated one from another and from other kinds of infectious agents; they can be used for extensive experiments conducted either *in vivo* or *in vitro*. Thus, to initiate the term virus used in connection with an infectious agent has lost its old indefinite meaning and has acquired a new significance similar in exactness to that borne by the words bacterium and spirochete. The terms virus of smallpox, *Virus variolae*, *Virus myxomatosum* (Sanarelli) and virus of poliomyelitis are now as definitive as are the terms bacillus of typhoid, *Bacillus typhosus*, meningococcus and staphylococcus. Such a statement does not imply that all viruses are alike in nature and that a subdivision of the viral group is not essential. The proper time for this subdivision, however, has not yet arrived.

Microorganisms were known to exist long before their relation to disease was appreciated. After the discovery of this relation it was not uncommon for more than one kind of organism to be accredited with the ability of producing the same malady. This fact is not surprising in view of the almost universal distribution of microbes. As early as 1840, before the specific relation of microorganisms to disease was accepted, Jacob Henle stated the conditions that should be met before an agent could be considered the proved cause of an infectious malady. Unfortunately, investigators were not guided by Henle's remarks, and it was necessary for Robert Koch to restate and emphasize them 40 years later.

In an article on the etiology of tuberculosis Koch in 1884 made the following statement:

The facts obtained in this manner can in every possible way serve as proof to which only extreme skepticism can still raise the objection

that the organisms found are not the cause but only concurrent phenomena of the disease. To be sure this objection often has a real justification and therefore it is not sufficient to establish only the concomitant occurrence of disease and parasite but the parasite must be shown to be the real cause. This can be done only by fully isolating the parasite from the body and all products of disease which might be considered as having a deleterious effect and producing the disease again with all its characteristics by the introduction of the isolated organisms into a normal host. (Author's translation.)

In 1890, speaking of bacteriological research before the Tenth International Congress of Medicine in Berlin, Koch expressed the same ideas in the following less mandatory manner:

However, if it can be proved: first that 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; secondly, that it occurs in no other disease as a fortuitous and non-pathogenic parasite; and thirdly, that it, after being fully isolated from the body and repeatedly grown in pure culture, can induce the disease anew; then the occurrence 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. (Author's translation.)

The above conditions laid down for the proof of the etiological relation of a microorganism to a disease constitute what are now known as Koch's postulates. His dictum has had a profound influence on workers investigating infectious maladies and for many years an infectious agent was not accepted as the cause of a disease unless the postulates had been satisfied. With the development of the science of immunology, however, immunological reactions added much to the knowledge of the specific relation of microbes to disease, and now it is possible to bring excellent evidence that an organism is the cause of a malady without the complete satisfaction of the postulates. In spite of this fact, there are certain workers who still refuse to agree that the cause of an infectious disease has been discovered unless all the conditions originally laid down by Koch have been met. This is particularly true regarding the viral maladies, the etiologi-

cal agents of which have not been cultivated on ordinary lifeless media.

It is unfortunate that so many workers have blindly followed the rules, because Koch himself quickly realized that in certain instances all the conditions could not be met, and in his paper before the Tenth International Congress of Medicine (1891), from which I have already quoted, the following statement occurs:

The proof has been fulfilled in a number of diseases, anthrax, tuberculosis, tetanus, and many animal diseases, in particular for almost all the diseases which are infectious for animals. Furthermore, it has been shown that in all cases in which it has been possible to demonstrate the regular and exclusive presence of bacteria in an infectious disease, the parasites never behave as accidental saprophytes but in the manner in which well known pathogenic bacteria act. Therefore, we are justified in stating that if only the first two conditions of the rules of proof are fulfilled, *i.e.*, if the regular and exclusive occurrence of the parasite is demonstrated, the causal relationship between parasite and disease is validly established. In accordance with this hypothesis we must then consider as parasitic a number of diseases in which it has not yet been possible—or only in an incomplete manner—to infect experimental animals and to prove the third part of the rules. To these diseases belong typhoid fever, diphtheria, leprosy, relapsing fever, asiatic cholera. In this connection I must mention cholera particularly because the inclusion of this as a parasitic disease has been opposed with unusual stubbornness. All conceivable efforts have been made to deprive cholera organisms of their specific character but they have withstood all attacks triumphantly and one can accept it as a generally confirmed and firmly grounded fact that they are the cause of cholera. (Author's translation.)

At the time when they were formulated Koch's postulates were essential for the progress of knowledge of infectious diseases; but progress having left behind old rules requires new ones which some day without doubt will also be declared obsolete. Thus, in regard to certain diseases, particularly those caused by viruses, the blind adherence to Koch's postulates may act as a hindrance instead of an aid. For instance, 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 old ideas, 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. Furthermore, it has been demonstrated that at least one natural disease of plants is induced by the combined action of two viruses, each of which has been obtained free from the other and when so obtained each produces a characteristic malady different from that caused by the synergistic action of the two agents.

The idea that an infectious agent must be cultivated in a pure state on lifeless media before it can be accepted as the proved cause of a disease has also hindered the investigations of certain maladies, inasmuch as it denies the existence of obligate parasitisms the most striking phenomenon of some infections, particularly those caused by viruses. Moreover, it ignores the possibility that certain viruses may be fabricated autocatalytically in living cells. One might say that the present-day method of propagating viruses in modified tissue cultures should be considered as taking the place of cultivation on lifeless media. I doubt whether the substitution is warranted, because the principles underlying the two methods of cultivation are radically different and Koch certainly did not have tissue-culture methods in mind when he proposed his rules of proof.

Koch's postulates are responsible for some odd conclusions regarding the cause of certain viral maladies. For example, a few investigators have claimed that streptococci are the inciting agent of poliomyelitis. Such claims, according to them, are based on the fact that Koch's rules have been satisfied. That is, streptococci have been found associated with the disease, they have been obtained in pure cultures from patients with the malady, they produce paralysis when injected into monkeys and rabbits, and they have been recovered in pure cultures from the experimental hosts. Furthermore, individuals recovering from poliomyelitis possess antibodies against the streptococci. To those unacquainted with the viral field and particularly to clinicians and bacteriologists unfamiliar with the pathological picture

of poliomyelitis, these claims seem valid. Consequently, they wonder why streptococci are not more generally accepted as the cause of infantile paralysis. The reason for lack of general acceptance is a simple one; the disease produced in the experimental animals is not poliomyelitis. Paralysis is not a characteristic sign of a single disease, and the pathological picture observed in the experimental hosts is quite different from that seen in human beings dead of infantile paralysis.

It is obvious that Koch's postulates have not been satisfied in viral diseases. Moreover, it is equally evident that proof of the etiological significance of viruses has been obtained without their satisfaction. Such a statement, however, does not imply that certain conditions do not have to be met before the specific relation of a virus to a disease is established. The conditions are: (a) A specific virus must be found associated with a disease with a degree of regularity. (b) 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.

In many respects the conditions just stated for viral maladies are similar to those of Koch for the proof of the specific relation of bacteria to disease. Nevertheless, there are certain differences. In the first place, it is not obligatory to demonstrate the presence of a virus in every case of the disease produced by it. Secondly, the existence of virus carriers is recognized. Finally, it is not essential that a virus be grown on lifeless media or in modified tissue cultures.

How does one go about proving that a virus is the cause of a disease? Viruses, regardless of whether they are parasites or the fabrications of autocatalytic processes, are intimately associated with host cells and, therefore, should always be found at the proper time in specific lesions. In addition, viruses, as is the case with bacteria, may be found also in the blood stream, not necessarily multiplying there but appearing frequently only as a phenomenon of overflow from lesions in the tissues. With these facts in mind, tissues with lesions, exudate from such lesions, and blood are collected aseptically and inoculated into a susceptible experimental host of the same or different species. The

material should be free from ordinary microbes; if not, the microbes should be killed or removed in a proper manner, *e.g.*, by filtration. If the inoculated animals become sick or die in a characteristic manner, and, if the disease in them can be transmitted from animal to animal by means of inoculations with blood or emulsions of involved tissues free from ordinary microbes or rickettsiae, one is fairly confident that the malady in the experimental animals is induced by a virus. On the other hand, such findings do not necessarily indicate that the active agent was present in the original material used for inoculation of experimental hosts.

When a natural disease under investigation exhibits characteristic features, *e.g.*, paralysis or intracellular inclusions, they are sought for in the experimental malady. If one finds them, one is encouraged, but proof is still lacking that the virus operating in the experimental hosts was present in the material taken from the individual with the natural infection. Not infrequently several viruses produce the same clinical and pathological pictures, and at times the same virus does not induce similar changes in different hosts. Consequently, regardless of the disease picture produced in the experimental animals, one is still faced with the problem of demonstrating that the virus causing it was present in the material used for inoculation of the first group of animals.

Experimental animals are subject to viral diseases of their own which may be encountered with sufficient frequency to cause mistakes. In this connection, I can speak from experience. At one time I thought that I had transmitted varicella to rabbits, for, when material from varicella patients was injected into rabbits and serial testicular passages were made, a virus that produced lesions similar to those observed in cases of human varicella was regularly obtained. However, later work in my laboratory and in that of Swift, involving injections of the virus into human volunteers, neutralization tests, and the discovery of the virus in stock rabbits, demonstrated conclusively that the virus with which I was working, now known as virus III, does not cause varicella in human beings, but produces a specific disease peculiar

to rabbits. My experimental animals were already carrying an agent capable of inducing lesions similar to those seen in varicella; the virus was activated by the experimental procedures.

Another example of the necessity of proving that a virus comes from a certain source is that encountered in our recent work on lymphocytic choriomeningitis. In this instance, the problem arose because the virus, with which we were working and which we believed came from sick human beings, is frequently found in mice under natural conditions. Furthermore, monkeys and guinea pigs are occasionally naturally infected. We were able, however, to show that our stock mice were entirely free from infection with this active agent, and it immediately became highly probable that we had actually isolated our virus from patients.

In addition to the fact that animals are subject to their own viral diseases which sometimes lead to confusion in the course of experimental work, they may become accidentally contaminated with an alien virus being studied in the laboratory to which they are susceptible. For instance, rabbits are highly susceptible to vaccine virus, and, if in this host serial testicular or cerebral passages, initiated by sterile broth, are made in a laboratory where the active agent is under investigation, it is almost impossible to avoid picking up the virus. This fact, which I have demonstrated more than once for my own satisfaction, most likely accounts for the ease with which certain Japanese workers seem to have isolated from human beings what they consider the specific viruses of varicella, measles and scarlet fever. In any event, the descriptions of the actions of their viruses and the intracellular changes observed in tissues infected with them are what one would expect to find as the result of a vaccinal infection. Thus, when several viruses are being studied in a laboratory, proper precautions must be employed to prevent the contamination of materials and animals used for the isolation of a virus from a newly recognized disease or for obtaining proof that a virus causes a clinical entity well known for many years.

Having demonstrated that a virus was obtained from an individual ill of a certain disease, one must then prove that the agent was actually causing the malady instead of occurring fortuitously

or instead of inducing a complicating or coexisting infection. There is no reason why individuals with poliomyelitis may not at the same time be affected with fever blisters; yet the virus of fever blisters recoverable from the patients is not responsible for their paralysis. When faced with such a situation, knowledge of the regularity with which a virus is associated with a given malady is of great assistance; if its presence is fortuitous or if it is the cause of a coexisting infection, it should not only be found irregularly in patients with the disease under investigation but should also be encountered under other conditions.

To illustrate the point in question certain facts about the development of knowledge of epidemic encephalitis or Economo's disease will be reviewed. Levaditi recovered a virus from a few cases of this malady, demonstrated that it produced an encephalitis in rabbits under experimental conditions, and, in spite of the fact that intranuclear inclusions were found in the brains of the rabbits while none were observed in human material, concluded that he had discovered the cause of the newly recognized infection of man. It remained for Blanc to demonstrate that Levaditi's virus was identical with one discovered a number of years previously and shown to be the cause of fever blisters. When this fact became known investigators registered doubt as to the etiological significance of Levaditi's virus in epidemic encephalitis. That doubt should arise is natural, because from a large number of cases only a few strains of the virus, ordinarily easily established in rabbits, were recovered. Furthermore, fever blisters is a common disease of man and many workers realized that its causative agent might occasionally be encountered accidentally in patients suffering from one of a number of maladies. Indeed, Flexner and Amoss searched for herpetic virus in the spinal fluid of patients with a variety of diseases and were rewarded for their trouble by finding it in the fluid of an individual with syphilis of the central nervous system. As a result of a great deal of work, most investigators are now of the opinion that Levaditi's virus or herpetic virus is not the cause of epidemic encephalitis, even though it has been recovered occasionally from the brain or spinal fluid of patients with the malady.

Knowledge regarding the regularity with which a virus is associated with a disease may be highly important, but 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. At the present time neutralizing antibodies are the most important, but complement-fixing antibodies, agglutinins and precipitins are being recognized more frequently in certain viral diseases and may eventually assume a significant place in experimental work on viruses.

Under at least two sets of conditions a virus of no etiological significance in certain diseases may occur in patients suffering from them. First, patients who have been affected previously by a viral disease continue as carriers after recovery to harbor the agent. Under such conditions they would possess antibodies against this virus at the beginning of their new illness as well as during convalescence. Secondly, it is conceivable that a virus might gain entrance into an individual and remain there only a short time causing little or no reaction. Under these circumstances, the virus, although capable of causing disease in experimental animals, would not incite the production of antibodies in the patients with the result that their serum would be devoid of antibodies both at the beginning and end of their illness. Some may doubt that this state of affairs occurs naturally. Nevertheless, it has been encountered not infrequently in experimental work.

If a virus is the actual cause of a disease, immune substances are usually absent from the patients' serum at the onset of illness and make their appearance during the period of recovery. However, this is not universally true, inasmuch as recovery sometimes takes place without the development of antibodies, and occasionally an individual possessing antibodies against a virus succumbs to a disease caused by it.

Although the absence of antibodies for a virus at the onset of an illness and their appearance later in the course of the disease or during convalescence constitute highly suggestive evidence that the virus is responsible for the malady, they alone should not be

accepted as incontrovertible proof that such is the case. The following example illustrates a striking exception to the rule. The Brown-Pearce tumor is a transplantable carcinoma of rabbits. Rabbits possessing no antibodies against virus III promptly and regularly develop them and become refractory to infection with the virus within two weeks after inoculation with the tumor. In view of these facts, one might on casual reflection conclude that virus III is the cause of the tumor. This is not true, however, because the virus alone does not cause the tumor, and the tumor freed from the virus does not produce in rabbits antibodies or resistance to the virus. In this case it is obvious that virus III, of no etiological significance so far as the tumor is concerned, is regularly carried in it, inciting the production of antibodies against itself in the carcinomatous animals.

To summarize, it can be said that the cause of viral diseases is known and that Koch's postulates as proposed by him do not have to be fulfilled in order to prove that a virus is the cause of a disease. However, the spirit of his rules of proof still holds in that a worker must demonstrate that a virus is not only associated with a disease but that it is actually the cause. The methods of doing this are different from the ones used by Koch but are equally efficient. At the present time, this is accomplished by the production with a degree of regularity of a transmissible infection in susceptible experimental hosts by means of inoculation of material, free from ordinary microbes or rickettsiae, obtained from patients with the natural disease, and by the demonstration through the use of proper controls and immunological studies described above that the virus was neither fortuitously present in the patients nor accidentally picked up in the experimental animals. Changes, notably the more extensive use of tissue-culture technics and serological reactions, will in the future undoubtedly occur in the methods of establishing the specific relation of viruses to disease; the number of changes will be limited only by the amount of ingenuity of investigators. 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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