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Chapter 7

The Discovery of the Causal Agent of the Tobacco Mosaic Disease

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ABSTRACT

The discovery of the causal agent of the disease causing mosaic and distortion on tobacco plants, with the concomitant realization that the etiologic agent was something unique - a virus - came at the end of the 19th century. This marked the beginning of the science of virology, although many diseases now known to be caused by viruses were described much earlier. This review documents the contributions of the three men who were the pioneers in this work, namely, Adolph Mayer, Dmitrii Iwanowski and Martinus Beijerinck.

To appreciate the discovery of the new infectious agent, the virus, at the end of the 19th century, we must think within the context of what was known about disease etiology at that time. It had only been appreciated for a short time that many diseases were caused by infectious entities. The work of pioneers like Pasteur, Lister and Koch had brought an appreciation of the causal agents of anthrax and tuberculosis. The dogma of the day was firmly entrenched in the postulates of Koch, who described in detail what was essential in order to establish the causal organism for a disease: 1) The organism must be associated with the pathological relationship to the disease and its symptoms; 2) The organism must be isolated and obtained in pure culture; 3) Inoculation of the organism from the pure culture must reproduce the disease; and 4) The organism must be recovered once again from the lesions of the host. A further and dominant consideration in the thinking of microbiologists was the belief that most infectious organisms would not pass through a filter of unglazed porcelain. Microbiologists and pathologists were confident that a microorganism would be identifiable for each infectious disease, and that it could be seen under the microscope.

The discovery saga of tobacco mosaic virus (TMV) begins with **Adolf Mayer**, Director of the Agricultural Experiment Station at Wageningen in The Netherlands. Mayer's attention was first called to study the peculiar disease of tobacco in 1879. Although known since the middle of the 19th

century in the literature, he is considered to be the first person to transmit tobacco mosaic by using the juice extracted from a diseased plant as the inoculum to infect other plants. His paper, published in 1886, in addition to describing the disease and its symptoms in detail, lists his attempts to ascertain the causal agent of the malady.

The disease which he named tobacco mosaic, was a serious impediment to tobacco growing, and he wrote that in some places it has "...caused the cultivation of tobacco to be given up entirely..".[1] He noted the symptoms of the disease, but as he observed healthy plants interspersed among diseased ones, he stated that "It may be accepted for certain, that an obviously diseased plant is never a source of infection for its surroundings." This observation contradicts current experience, and is also at variance with his own transmission studies.

Adolf Mayer, 1843-1942

Mayer's studies encompassed a comparative chemical analysis of healthy and diseased leaves to see if a difference in nutrition could explain the disease. Similarly, analyses of soil in which diseased plants were grown and the presence of nematodes in those soils failed to reveal the cause. He investigated temperature, light, fertilization, looked for fungi or "animal parasites". "Then I suddenly made the discovery that the juice from diseased plants obtained by grinding was a certain infectious substance for healthy plants." This exciting discovery prompted a careful look at the inoculum source for "protoplasmic bodies ...taken up with special zeal." He tried to follow Koch's postulates, and indeed, was able to culture organisms from the extracts, but of course, none of these would reproduce the disease. He tried to reproduce the disease by inoculation with a number of well-known bacteria and fluids, such as manures from several animals, including man, grated old cheese and putrefied legumes. He was left with the conclusion that the infectious agent was either an enzyme, or some sort of microorganism. He thought it absurd to think it was an enzyme, as it would not be able to reproduce itself. He did experiments with filter paper, and found the agent passed through initially, but upon repeated filtration, in which a "clear filtrate" is obtained, the extract was not infectious, and concluded that the infectious agent was a bacterium. (He ruled out fungi, as he considered that they would not pass through even the first filtration with paper.) This is a strange result, as with current experience with TMV, one would not expect that it would be

retained by filter paper; it takes nitrocellulose or similar filters of very small pore size to retain it. Although Mayer came to the wrong conclusion about his finding, he died in 1942 at age 99 which would have given him adequate opportunity to see the modern concept of the virus develop, including its purification of the virus by Stanley in 1935.

The man most often given credit for the discovery of the nature of the infectious agent both in the past (Stanley, 1944) and recently (Lustig and Levine, 1992; Levine, 1966) was **Dmitrii Iwanowski** (sometimes transliterated as Ivanowski or Ivanovsky). However, as considered below, a recent polemic (Horzinek, 1995) suggests Martinus Beijerinck should be given credit (Bos, 1995), as in contrast to Iwanowski, he appreciated the significance of his findings.

Dmitrii Iwanowski 1864-1920

Iwanowski presented his findings to the Academy of Science in St. Petersburg (Russia) in 1892. He disputed the findings of Mayer with respect to the filterability of the agent of tobacco mosaic disease through double filter paper. He did a number of filtration experiments of his own, using porcelain Chamberland filter-candles, which were considered to be ultimate test for bacteria, as they would be retained on the filter. He was surprised by the result and suspected a defective filter could explain the agent's filterability, but convinced himself by further testing of the filters that they were not defective.

"According to the opinions prevalent today, it seems to me that the latter is to be explained most simply by the assumption of a toxin secreted by the bacteria present, which is dissolved in the filtered sap. Besides this there is another equally acceptable explanation possible, namely, that the bacteria of the tobacco plant penetrated through the pores of the Chamberland filter-candles, even though before every experiment I checked the filter used in the usual manner and convinced myself of the absence of fine leaks and openings."[1]

Iwanowski had one final publication on TMV in 1903. In contrast to his earlier very short paper, this one was a detailed description of the disease, including microscopic observations on the two types of inclusions found in cells of infected tissues, and made extensive unsuccessful efforts to culture the agent. Nevertheless, he still came to the conclusion that the causal agent was an unculturable bacterium. He was aware, however of the report of Beijerinck, but that did not sway him from the above conclusion.

The article by **Martinus Beijerinck**, published in 1898 is very insightful

and is the most detailed on the disease and its causal agent. Of the three reports considered here, it is by far the most detailed and innovative in its approach to the study of the tobacco mosaic disease.

Martinus Beijerinck, 1851-1931

Beijerinck looked for "microbes" associated with the disease, and could find none. He repeated the filtration experiments of his predecessors (although he was apparently unaware of the work of Iwanowski according to Bos [1995]) and concluded that what passed through porcelain filters remained infectious and was sterile of microorganisms. He concluded that it was a "contagium vivum fluidum", a contagious living fluid. He found that from the sap of a diseased plant, "..an infinite number of healthy plants may be inoculated and infected.." and concluded that the infectious agent reproduces itself in the diseased plants. To demonstrate that the infectious agent was not a microbe, he conducted diffusion experiments, in which he allowed the "virus" to penetrate an agar plate. He found that indeed it could penetrate "..to no small depth." He found that the agent diffused about 2 mm in 10 days, and concluded that the virus was soluble, which indeed it is. He found that the infectious extract was stable during a three month test, but the virulence did not increase or decrease in the extract, further evidence that it was not bacterial. He also observed that the virus remains viable, without loss "..in strength of infection.." even after the tissue is dried, but that heating the extract to 90^{o} inactivated it.

Beijerinck studied the disease itself in detail, using his capacity to transmit it as a means of deciding the presence or absence of virus. He concluded, based on symptomatology, that only young leaves could be infected, and concluded that only cells which are dividing can become infected - certainly not the case, as it is now known that old leaves may become infected, but rarely show symptoms. He correctly deduced that the virus moved within the plant in the phloem, although he felt that occasionally it also moved in the xylem, a notion that is sometimes debated today.

Bos (1995) contends that credit for the discovery of TMV as a virus should go to Beijerinck. He argues that even in his exhaustive 1903 paper, based on his thesis, Iwanowski did not appreciate the significance of his finding. He still argued in favor of a bacterial etiology, and felt that spores of the causal organism were able to pass through the filter. Beijerinck, on

the other hand, did appreciate that he has something quite different from microbes and Bos feels that for that reason, he deserves credit for developing the viral concept.

Nevertheless, whomever is the one history will record as the discoverer of the virus concept, these three gentlemen are to be remembered for their insights into the investigations to find the causal agent for a puzzling disease. Tobacco mosaic virus has been very important in the history of virology for many reasons beyond the discovery of the viral concept. It was the first to be purified (Stanley, 1935), and many fundamental concepts of virology were developed with it. The chemical composition of the virus, the isolation of its protein and nucleic acid components, its immunogenicity, infectious RNA, reconstitution from its dissociated parts, the first determination of the sequence of a viral coat protein are among the milestones. These are chronicled in an article by Heinz Fraenkel-Conrat (1986), who himself made many of those discoveries.

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[1]Quotations are from the English translations by James Johnson of the papers of Mayer, Ivanowski and Biejerinck.