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The embryonic beginning of virology: unbiased thinking and dogmatic stagnation

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Introduction

Occasions, such as the recent appearance of the Encyclopedia of Virology, provide an opportunity to reconsider the discipline's roots and to critically reexamine the past. In plant pathology, Mayer [20], Ivanovsky [9] and Beijerinck [1] have, for their classical investigations on tobacco mosaic published between 1886 and 1898, for long been considered to have set the stage for virology as a new discipline, and to have done so together [12]. However, a minireview on *"One Hundred Years of Virology"* that was published in 1992 [18] fixed the beginning of virology to 1892, and claimed Ivanovsky's role "as the father of the new science of Virology". The review must have served as a prelim to the *"Foreword: 100 Years of Virology"*, published two years later in the new Encyclopedia of Virology [17]. The foreword affirmed Ivanovsky's *"priority to the discovery of viruses"* and his key-role in the history of the science covered by the encyclopedia, and hailed this exemplary *"pioneering spirit"*. Since a number of data conflict with historical fact, a 'letter' on the matter has recently been submitted and meanwhile accepted for publication [5], and the present article now provides some further documentation.

Ivanovsky

Ivanovsky is usually quoted for his classical filtration experiments demonstrating passage of the causative agent of tobacco mosaic through the pores of a bacteria-proof Chamberland filter. His paper, read before the Academy of Sciences in St. Petersburg, Russia in 1892 [9], is a landmark in the history of virology. Of special significance, however, is Ivanovsky's commonly ignored dissertation published in German in 1903 while he was working in Warsaw [11]. Lustig and Levine [18] have obviously studied that paper since they refer to it when mentioning Ivanovsky's work on the inclusion bodies in the host cells of virus-diseased plants [17]; however, they overlooked its last, historically most important section on *"the culture of the microbe of the mosaic disease"*. Peculiarly enough, the same had been done by Johnson (1942) in his biographical sketches accompanying the English translations of the papers by Mayer, Ivanovsky and Beijerinck [12], published in the Phytopathological Classics of the American Phytopathological Society.

From the beginning Ivanovsky had kept insisting that he was dealing with a microbe that might have passed the pores of the bacteria-proof filter or might have produced a filterable toxin [9]. In reaction to Beijerinck's report, he related in 1899 that by 1892, he had "succeeded in evoking the disease by inoculation of a bacterial culture, which strengthened my hope that the entire problem will be solved without such bold hypotheses" [10]. Kluyver, Beijerinck's successor in Delft, later wrote that "anybody reading Ivanovsky's 1899 paper will have to acknowledge that this author, even seven years after he made his discovery, was not at all aware of its tremendously far-reaching importance, the main part of the paper being devoted to an attempt to prove contrary to all available evidence the bacterial nature of the contagious agent" [14]. In 1903, when further criticizing Beijerinck's conclusion about the *contagium vivum fluidum* [1], he claimed it to be a *contagium vivum fixum*. He wrote that "the persistence of infectivity of the filtered sap can only be explained by the assumption that the microbe produces resting forms, that is spores". He found that, like particles of Indian ink, the contagium could pass through agar, and in the last paragraph of his 1903 paper he categorically concluded *"that the contagium of the mosaic disease is able to multiply in the artificial media"*. This clearly demonstrates that Ivanovsky did not grasp the scope of his observations. *"It shows the outcome when theory (Koch's Postulates) fossilizes into dogma"* [4].

Hence, the decisive questions are

- whether Ivanovsky should really be considered the *"first discoverer of viruses"*, as suggested by the postage stamp issued on the occasion of his 100th birthday (Fig. 1),
- whether his filtration experiments should be marked as *"the first step in the discovery of viruses"*, and
- whether in 1992 virology could be judged to have *started 100 years ago* [17, 18].

Fig. 1. Postage stamp issued in the USSR in 1964 on the occasion of the 100th birthday of Dimitrii Ivanovsky (1864–1920)

Mayer and Beijerinck

Earlier, in the Netherlands, Mayer (Fig. 2) had entered the scene of phytopathology. Originally a German agricultural chemist of the Liebig school, he was appointed professor in Wageningen in 1876. He was soon questioned about the enigmatic mosaic disease of tobacco, then prevalent in the region and he first reported on it in 1882, unfortunately only in Dutch [19], naming it "tobacco mosaic". In his classical publication of 1886 ([20], in German), Mayer concluded that the "answer to the question submitted to us is not to be found in the field of nutrition". Also, in inoculation experiments none of the bacteria, obtained when trying to isolate "organisms according to Koch's methods and other methods", were infectious. However, "if one grinds up finely a leaf that is clearly diseased with a few drops of water and sucks the thick green emulsion that is thus obtained into fine capillary glass tubes and then sticks these into the thick leaf veins of an older plant ... in nine cases out of ten one will be successful in making the healthy plant ... diseased". Mayer is therfore credited for proving the infectious nature of the disease in the apparent absence of microorganisms. Particularly noteworthy is his 1882 speculation on the existence of *"possibly a soluble ('enzyme'-like) contagium, although almost any analogy for such a supposition is lacking in science"* [19]. In his best-known 1886 paper [20] he did not follow up on this idea "because an attempted isolation led to no preparation capable of producing infection". He finally concluded that the mosaic disease "is bacterial, but that the infectious forms have not yet been isolated, nor are their forms and mode of life known" [20].

The 1880s and early 1890s, dominated by the gradually emerging Postulates of Koch, were not yet ripe for thinking of pathogens other than of "germs" that could be cultivated on artificial media. In 1885, Beijerinck (Fig. 3), teacher of botany at the Agricultural School where he also had become intrigued by tobacco mosaic, left Wageningen to take a position as microbiologist of the Netherlands Yeast and Alcohol Factory in Delft. At the request of Mayer,

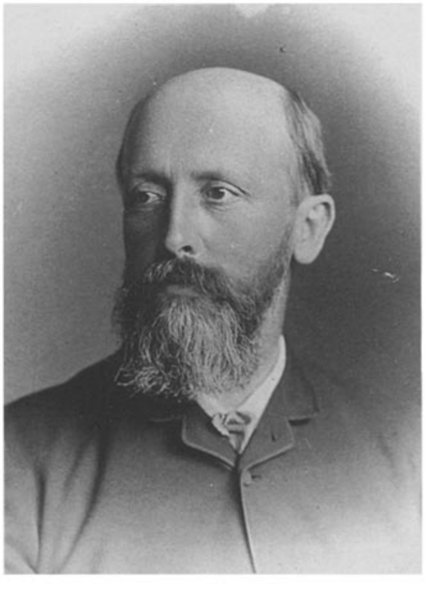

Fig. 2. Photograph of Professor Adolf Mayer (1843–1942), taken during his active career (Historical Collection, Agricultural University, Wageningen)

he had tried to isolate the responsible microorganism. The successful isolation of the root-nodule organism encouraged him in 1887 to make another attempt to isolate the tobacco mosaic agent – but again in vain [14]. Soon after he had become professor of microbiology at what is now the Delft Technical University, Beijerinck began more systematic investigations on tobacco mosaic which led to his classical 1898 paper, published in Dutch and German [1]. Unaware of Ivanovksy's five years earlier report [9] he also filtered sap from diseased tobacco plants through porcelain, found it to be (bacteriologically) sterile, and observed that very small quantities of filtrate were infectious. From such plants again a large number of plants could be infected and he came to conclude that "the contagium reproduces itself in the living plant". He proved that it multiplied only in tissue in which cell division occurred and that it precipitated from aqueous solution without loss of infectivity. When reading Beijerinck's 1898 publication, one must conclude that he was the first to internationally voice the novel nature of the agent causing tobacco mosaic, completely different from microbial corpuscular organisms. When he claims that "the infection is not caused by a microbe, but by a *contagium vivum fluidum*", thence in his paper called a virus, he specifies it to be a "liquid or soluble agent" that "reproduces itsef in the living plant".

Beijerinck's observations and considerations were obviously linked to and based upon Mayer's. In the light of developments during the 1920s, leading to the final purification by Stanley (1935) of tobacco mosaic virus as a chemical substance, Mayer's 1882 allusion to the *"possibly soluble (enzyme-like) nature"* of the contagium of tobacco mosaic is remarkable. The spirit of the time and the lack of molecular insight did not allow this perception to take off and to revolutionize scientific thinking. But towards the end of the 19th century there was an obvious dawn of change.

Fig. 3. Martinus Willem Beijerinck (1851–1931), teacher of botany at the Agricultural College in Wageningen from 1876–1885, then professor of microbiology at the Polytechnical School, later Technical University, of Delft (1895–1921) (Historical Collection, Agricultural University, Wageningen)

Dawn of change and stagnation

During the last one or two decades of the 19th century the awareness of something new became tangible. In addition to tobacco mosaic there was a growing number of plant diseases that did not conform with Koch's rules.

Monumental were Erwin F. Smith's extensive investigations in the USA on peach yellows, a non sap-transmissible plant disease reported in 1888. Smith could transfer the disease by budding or grafting, but was not able to reveal an incitant [21]. In 1893/94 interesting phenomena, now known to be related to the symptoms of peach yellows were studied in Amsterdam by the famous Dutch botanist and geneticist Hugo de Vries [7]. They included an "epidemic of virescences", teratological floral abnormalities including greening (virescence) and phyllody of floral organs [2], characteristic of aster yellows, later to be studied in detail in the USA [16]. He found 27 plant species affected in his garden, but could not discern a cause, neither macroscopically nor microscopically, and therefore hesitated to publish his observations. When he finally did, he was *"convinced of the infectious nature of the disease and of its spread by flying insects, in the hope that others may later be more successful in finding the parasite"* [7].

Most of the agents of such diseases were later indeed found to be transmitted by leafhoppers, as first reported for aster yellows in the 1920s [15, 16]. The agents did meet the definition of a virus then prevailing. That is why a paper presented during a conference in commemoration of Ivanovsky's 100th birthday (Moscow, 1964), could bear the title "Hugo de Vries and early plant virology" [3]. It took until 1967 before electron microscopy of ultra-thin sections of diseased vascular tissue revealed the involvement of minute filterable mycoplasma-like organisms (*Mollicutes*) inhabiting the phloem [8]. The discovery and further study of pathogenic mycoplasmas have thus contributed to a definition and better unterstanding of the nature of viruses.

In virology, developments have been slow. In 1882, there was a first dawn of change, thanks to unbiased thinking, with new momentum in 1898, but the paper on "the culture of the microbe of the mosaic disease" [11] illustrates that there was also dogmatic stagnation.

Embryonic start

Analogies for Mayer's and Beijerinck's new ideas were, in Mayer's own words [19], "still lacking in science" at the turn of the century. From today's point of view, and surveying contemporary theory, their ideas must be qualified as revolutionary. Raemaeker's remarkable cartoon of Mayer (Fig. 4), drawn probably on the occasion of Mayer's retirement in 1904 from the then Agricultural College at Wageningen, is testimony of Mayer's colleagues' apprehension that he was dealing with something "at the threshold of life". The cartoon also symbolizes the embryonic beginning of virology in 1882, considerably earlier than Ivanovsky's and Beijerinck's findings. Mayer deserves credit for having developed a revolutionary idea and to have set the stage for Beijerinck's classical research and theory.

A long period of stagnation followed. An essential step was taken in the 1930s with Stanley's isolation in 1935 of tobacco mosaic virus from diseased plants [22] and its observation with the electron microscope in 1939 [13]. The discovery of Mollicutes and later of viroids have helped to focus the virus definition [4]. The foundations to all these discoveries were laid as early as in 1882 and have been celebrated in a symposium on

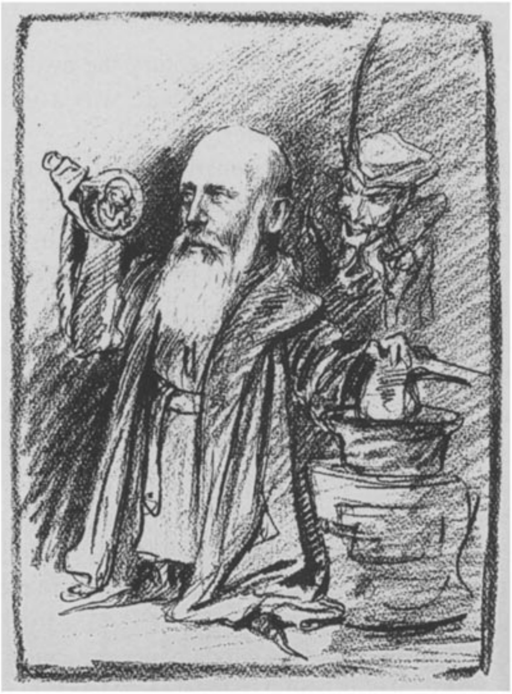

Fig. 4. Cartoon of Professor Adolf Mayer about 1904 by Louis Raemaekers, then teacher of drawing at the Agricultural College and later known for his political cartoons. Mayer is depicted as Goethe's Dr. Faust with Mephistopheles as the symbol of evil in the background. It dramatically illustrates the awareness about Mayer's involvement in phenomena at the threshold of life and the hesitancy about the possible Promethean outcome of modern Cartesian science. The cartoon also symbolizes the embryonic beginning of virology (Historical Collection, Agricultural University, Wageningen)

"100 years of virology at Wageningen" by the Agricultural University and the Research Institute for Plant Protection (IPO-DLO) [6].

Conclusion

Mayer, Ivanovsky, and Beijerinck were all pioneers of virology, but none of them knew what he was to expect. The trained chemist Mayer [19] was close to the mark, and Ivanovsky [9] made an important discovery later. Unfortunately, he not only "failed to understand the full significance of his filtration experiments", as admitted by Lustig and Levine [18], but stuck to prevailing dogma and refused to grasp the implications of his findings, later going completely astray [10, 11]. Literally he was right, however, in that the contagium was particulate, as revealed much later. The pathogen is now known to be truly a *contagium vivum fixum*, as Ivanovsky kept insisting, but in a different sense than the organismal corpuscularity he had in mind. Beijerinck's claim of a "fluidity" of the contagium is a matter of semantics since he already used it interchangeably with "solubility". His coining of the term "virus" for a new class of pathogens further marks the beginning of a new era in biology.

Hence, it is not very fertile to debate the issue who was first in discovering a virus. None of the pioneers could have known what he was talking about. What counts is the spirit in which new experimental findings were faced. Scientific evolution requires independent, unbiased thinking; dogmatic adherence to current theory implies stagnation. Is it *"the spirit of Ivanovsky"*, acclaimed in the Encyclopedia of Virology [17], that *"is still required of his successors to meet the challenges of virus evolution"*, or rather, of the evolution of virology? I leave it to the reader to decide whether Ivanovsky must be identified as the intellectual and conceptual father of virology as a new field of science.

References

1. Beijerinck MW (1898) Über ein *contagium vivum fluidum* als Ursache der Fleckenkrankheit der Tabaksblätter. Verh Kon Akad Wetensch 65 (Sectie 2, Deel 6): 3–21 (Engl. translation: Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves. Phytopath Classics 7 (1942): 33–54)
2. Bos L (1957) Plant teratology and plant pathology. Tijdschr Plantenz 63: 222–231
3. Bos L (1966) De Vries and early plant virology (in Russian). In: Protsenko AE , Rischkov VI (eds) On the nature of viruses. Proc Conf 100th birthday Ivanovsky, Moscow, October 1964: 52–54
4. Bos L (1981) Hundred years of Koch's Postulates and the history of etiology in plant virus research. Neth J Plant Pathol 87: 91–110
5. Bos L (1995) One hundred years of Virology? ASM News 61 (in press)
6. De Bokx JA, de Jager CP, Noordink JPW (eds) (1982) 100 Jaar Virologie in Wageningen (in Dutch: 100 Years of Virology in Wageningen). Gewasbescherming 13: 135 pp
7. De Vries H (1896) Een epidemie van vergroeningen (in Dutch: An epidemic of virescences). Bot Jaarb Gent 8: 66–91
8. Doi Y, Teranaka M, Yora K, Asuyama H (1967) Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows or *Paulownia* witches' broom (Japanese with Engl. summary). Ann Phytopath Soc Japan 33: 259–266
9. Ivanovsky D (1892) Über die Mosaikkrankheit der Tabakspflanze. St. Petersb Acad Imp Sci Bull 35: 67–70 (id Beih Bot Centralbl 3 (1893): 266–268) (Engl. translation: Concerning the mosaic disease of tobacco. Phytopath Classics 7 (1942): 25–30)
10. Ivanovksy D (1899) Über die Mosaikkrankheit der Tabakpflanze. Centralbl Bakteriol II 5: 250–254
11. Ivanovsky D (1903) Über die Mosaikkrankheit der Tabakpflanze. Z Pfl Krankenh 13: 1–41
12. Johnson J (1942) Translator's preface and translations with short biographies of Mayer, Ivanovsky, Beijerinck and Bauer. Phytopath Classics 7: 62 pp
13. Kausche GA, Pfankuch E, Ruska H (1939) Die Sichtbarmachung von pflanzlichem Virus im Übermikroskop. Naturwissenschaften 27: 292–299
14. Kluyver AJ (1940) Beijerinck, the microbiologist. In: Van Iterson G, Den Dooren de Jong LE, Kluyver AJ (eds) Martinus Willem Beijerinck, his life and his work. Nijhoff, The Hague, pp 99–154
15. Kunkel LO (1924) Insect transmission of aster yellows. (Abstr) Phytopathology 14: 54
16. Kunkel LO (1926) Studies on aster yellows. Am J Bot 13: 646–705 (id Contr Boyce Thompson Inst 1 (1926): 181–240)
17. Levine AJ, Lustig A, Lvov DK (1994) Foreword: 100 years of virology. In: Webster RG, Granoff A (eds) Encyclopedia of Virology. Academic Press London I: XLI–XLIII
18. Lustig A, Levine AJ (1992) Minireview: One hundred years of virology. J Virol 66: 4629–4631
19. Mayer A (1882) Over de mozaikziekte van de tabak; voorloopige mededeeling (in Dutch: On the mosaic disease of tobacco; preliminary communication). Tijdschr Landbouwk 2: 359–364
20. Mayer A (1886) Über die Mosaikkrankheit des Tabaks. Landw Vers Stn 32: 451–467 (Engl. translation: Concerning the mosaic disease of tobacco. Phytopath Classics 7 (1942): 11–24)
21. Smith EF (1888) Peach yellows, a preliminary report. Bull Div Bot US Dep Agric 9: 1–254
22. Stanley WM (1935) Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. Science 81: 644–645

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