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THE ROYAL SOCIETY

Beijerinck's work on tobacco mosaic virus: historical context and legacy

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Beijerinck's entirely new concept, launched in 1898, of a filterable *contagium vivum fluidum* which multiplied in close association with the host's metabolism and was distributed in phloem vessels together with plant nutrients, did not match the then prevailing bacteriological germ theory. At the time, tools and concepts to handle such a new kind of agent (the viruses) were non-existent. Beijerinck's novel idea, therefore, did not revolutionize biological science or immediately alter human understanding of contagious diseases.

That is how bacteriological dogma persisted, as voiced by Loeffler and Frosch when showing the filterability of an animal virus (1898), and especially by Ivanovsky who had already in 1892 detected filterability of the agent of tobacco mosaic but kept looking for a microbe and finally (1903) claimed its multiplication in an artificial medium. The dogma was also strongly advocated by Roux in 1903 when writing the first review on viruses, which he named 'so-called "invisible" microbes', unwittingly including the agent of bovine pleuropneumonia, only much later proved to be caused by a mycoplasma. In 1904, Baur was the first to advocate strongly the chemical view of viruses. But uncertainty about the true nature of viruses, with their similarities to enzymes and genes, continued until the 1930s when at long last tobacco mosaic virus particles were isolated as an enzyme-like protein (1935), soon to be better characterized as a nucleoprotein (1937). Physicochemical virus studies were a key element in triggering molecular biology which was to provide further means to reveal the true nature of viruses 'at the threshold of life'.

Beijerinck's 1898 vision was not appreciated or verified during his lifetime. But Beijerinck already had a clear notion of the mechanism behind the phenomena he observed. Developments in virology and molecular biology since 1935 indicate how close Beijerinck (and even Mayer, Beijerinck's predecessor in research on tobacco mosaic) had been to the mark. The history of research on tobacco mosaic and the commitments of Mayer, Beijerinck and others demonstrate that progress in science is not only a matter of mere technology but of philosophy as well. Raemaekers' Mayer cartoon, inspired by Beijerinck, artistically represents the crucial question about the reliability of our images of reality, and about the scope of our technological interference with nature.

Keywords: tobacco mosaic virus; virus discoveries; Koch's postulates; history of (plant) virology; concepts in virology

1. INTRODUCTION

To appreciate fully the tremendous progress achieved in studying tobacco mosaic virus (TMV) for a period of 100 years and to evaluate its impact on virology in general and on molecular biology, it seems appropriate to look more closely for the roots of the discipline as well as for the legacy of pioneers, such as Martinus Willem Beijerinck, who were involved in its birth. Progress in science is not a matter of mere *technology*, but of *philosophy* as well; that is, the involvement of the human mind and of humans in their perception of life and reality. Progress, therefore, is reflected in terminology, and in the definition of terms, that is in human *concepts*, ideas, theories, or *images of reality*. Within a discipline, concepts evolve with time, or change radically when current theory fails to explain hitherto overlooked or ignored phenomena. That is how Beijerinck (figure 1)—when reading his classical paper 'Concerning a contagium vivum fluidum as cause of the spot disease of tobacco leaves' before the Academy of Sciences in Amsterdam (Beijerinck 1898*a*) and claiming that bacteriological methods already applied in 1887 had failed to reveal the cause of the disease—was led to conclude 'that an example was found of disease, caused by a contagium which does not match the *concept* [author's emphasis] connected with the contagium fixum' (that is, corpuscular bacteria). Thus a conceptual change took place in 1898, but obviously something had been in the air already. Before dwelling on Beijerinck's legacy for science, we must, therefore, first examine its historical perspective (see also Smith Hughes 1977; Waterson & Wilkinson 1978).

2. DOMINANCE OF BACTERIOLOGY

For the historical context of Beijerinck's work we must go back to the middle of the 19th century. *Vitalism* (claiming life to be more than mere physicochemistry) and belief in *spontaneous generation* (considering micro-organisms to be a result rather than cause of disease), ideas which had for long dominated biology and man's views about nature, began to lose ground. In agriculture,

Figure 1. Martinus Willem Beijerinck (1851–1931). Photograph taken at the age of 45. (The image is taken from an album presented to Mayer at his retirement in 1904, and is reproduced with permission of the Historical Collection, Agricultural University, Wageningen.)

Albrecht Thaer's humus theory had to give way to von Liebig's theory on remineralization of organic matter and on the prime role of mineral elements in plant nutrition (Liebig 1840). Agricultural *chemistry* started boosting agricultural production and stimulated agricultural research. Louis Pasteur (1860) demonstrated that life does not originate spontaneously but needs germs from which to develop. Robert Koch (1876), when studying anthrax of cattle, was the first to show convincingly that contagious disease results from infection by micro-organisms which can be cultivated in or on artificial media and can be back-inoculated into disease-free specimens of the natural host to reproduce disease. This is how *microbiology* (or better, *bacteriology*) emerged as a new discipline.

After Koch's *in vitro* cultivation of *Bacillus anthracis* (1876), *Mycobacterium tuberculosis* (1882), and *Vibrio cholerae* (1883), to mention a few examples, microbe hunting was really on. Solid media (developed in 1880) were of great help. Important ailments of plants were likewise linked to the presence of bacteria that could be grown *in vitro*. Early examples are *Erwinia amylovora*, the cause of fire blight of apple and pear (Burrill 1880, 1886), and *Xanthomonas hyacinthi*, the agent of 'yellow disease' of hyacinth (Wakker 1883). Such successes drew widespread interest and led to establishment of the *Institut Pasteur* in Paris (in 1888) and the *Institut für Infektionskrankheiten* in Berlin (in 1891; with Koch as its first director), further promoting the new field of science.

Thus there was rapid progress. However, Koch's successful methods, which gradually became known as *Koch's Postulates*, turned into a pervasive theory; bacteriology began dominating the study of disease and the postulates were even converted into *dogma*: viruses—a term hitherto widely used for any poisonous or venomous disease-inciting agent—*are always microbes* (Pasteur 1890: 'tout virus est un microbe'). But towards the end of the century there was change in the air because there were phenomena conflicting with the then current theory.

3. PRELUDE TO VIROLOGY: MAYER

For the scene where plant virology had its major roots we must go to Wageningen, a small country town in the centre of The Netherlands. In 1876, an Agricultural School (nowadays Wageningen Agricultural University, WAU) was established there. Beijerinck (1851–1931), a young botanist originally trained in chemistry, was appointed Teacher in Botany. Adolf Eduard Mayer (1843–1942) (with the personal title of Professor), a German graduate from the school of Liebig, eight years younger than Beijerinck, with expertise in agricultural chemistry (Mayer 1870), was appointed to teach agricultural chemistry and to direct the Agricultural Experiment Station (nowadays the Agricultural Research Service, DLO) which was then affiliated with the school. The two teachers were on good terms, and to maintain a scientific attitude in the somewhat isolated town in 1876, the year of their appointment, they established the still extant local Natural Science Society (Mayer 1931).

In 1879, soon after coming to Wageningen, Mayer (figure 2) was requested by farmers in the region west of the town, where tobacco was an important crop, to study a tobacco disease that was prevalent then. He called it mosaic disease (figure 3), and despite the lack of evidence of causal involvement of a visible organism, nutritional factors, humidity, or temperature, Mayer soon proved its infectious nature by transfer of the causal agent in expressed sap which was introduced into healthy plants by pricking with glass capillaries. When first publishing on the disease in an often overlooked paper, Mayer (1882) speculates with remarkable originality on the existence of a 'soluble, possibly *enzyme-like contagium* [author's emphasis], although almost any analogy for such a supposition is failing in science'. In this respect, it is of interest that at that time Mayer had already written a handbook on fermentation (Mayer 1879). In his classical paper on tobacco mosaic, published in German in 1886, he unfortunately gives up the idea of the possible involvement of an enzyme and largely sticks to the prevailing theory, however, with the interesting restriction that the mosaic disease 'is bacterial, but that the infectious forms have not yet been isolated, nor are their forms of life known' (Mayer 1886). Beijerinck (1898) later narrates that when at that time his colleague Mayer showed him his experiments, he (Beijerinck) also was unable to detect microbes to which the disease could be ascribed, but admits that his bacteriological knowledge was then incomplete.

Figure 2. Professor Adolf Mayer (1843–1942). (The image is taken from an album presented to Mayer at his retirement in 1904, and is reproduced with permission of the Historical Collection, Agricultural University, Wageningen.)

4. THE THRESHOLD OF CHANGE: BEIJERINCK

In Wageningen, Beijerinck devoted a considerable part of his time to important research, for example, on plant galls and cereal breeding. In 1877, his first important paper dealt with plant galls (Beijerinck 1877). It was elaborated that same year into a dissertation submitted for a PhD degree in Utrecht. The outstanding results enabled him to qualify for membership of the Netherlands Royal Academy of Sciences in 1884, at a comparatively early age.

Beijerinck left Wageningen in 1885 for the highly productive microbiological part of his career that would bring him world fame. At that time he was appointed head of the bacteriology laboratory of the Yeast and Spirits Factory (the present Gist-Brocades Company) at Delft, between The Hague and Rotterdam. He also found time and was permitted to pursue personal interests, which led, in 1887, to his isolation and *in vitro* cultivation of the root-nodule bacterium *Bacillus* (now *Rhizobium*) *radicicola* of the Leguminosae (Beijerinck 1888). This success, he wrote in 1898, encouraged him to resume the study of tobacco mosaic. Since attempts to isolate a causal bacterium failed again, and this also held for anaerobic bacteria painstakingly sought in leaves, roots and surrounding soil, he then concluded that a *contagium fixum* could not be causally involved (as noted in § 1).

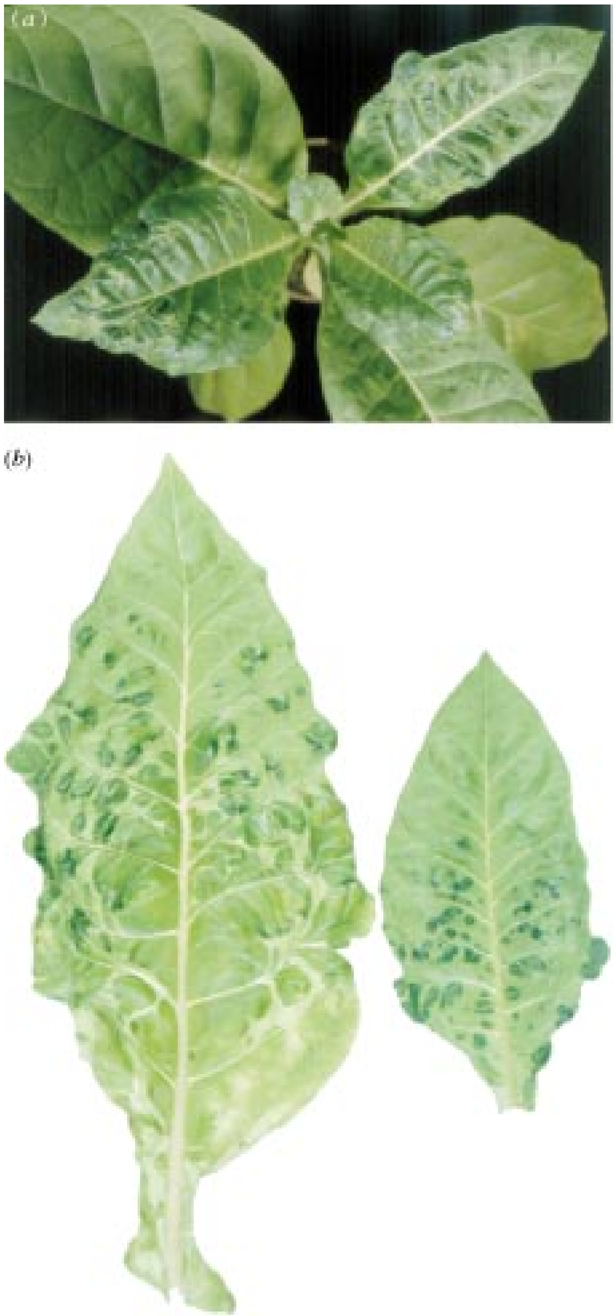

Figure 3. Symptoms of tobacco mosaic virus in whole plant (*a*) and detached leaves (*b*). (Photograph: IPO-DLO, Wageningen.)

In 1895, at the age of 45 when his well-known photograph (figure 1) must have been taken, the more academic phase of Beijerinck's career began when he was appointed Professor of Bacteriology at the Polytechnical School (now Technical University) at Delft. When, two years later (in 1897), a new bacteriology laboratory and greenhouse were built, he immediately commenced the series of decisive experiments that would lead to the classic but preliminary paper read before the Royal Academy on the 26th of November 1898 (Beijerinck 1898*a*). An enlarged account in German, printed soon thereafter in the same proceedings (Beijerinck 1898*b*, 1899*a*) led to wide publicity, further enhanced by a French version with a postscript issued in 1900 (Beijerinck

1900*a*). An English translation, of the German text appeared in 1942 in the American Phytopathological Society's *Phytopathological Classics* (together with translations of the papers by Mayer (1886), Ivanovsky (1892) and Baur (1904), with a foreword by the translator James Johnson (1942)) and later in Hahon's *Selected papers on virology* (Hahon 1964).

Beijerinck's momentous document on tobacco mosaic still makes fascinating reading for its clarity of language and reasoning. A significant aspect was the application of unglazed filter candles—developed 13 years before by Chamberland (1884) to obtain 'physiologically pure' water—for removing all visible micro-organisms from expressed plant sap. The major observations were as follows:

1. Crude extracts from diseased plants passing through porcelain filter candles do not show bacterial growth during three months of storage, but remain infective. Subsequent plant inoculation by injection readily leads to infection and reproduction of the characteristic symptoms.
2. Unlike bacteria, the infectious agent diffuses laterally into agar for at least 2 mm.
3. The agent multiplies in plants, as shown by serial transfers from plant to plant, and cannot be a toxin.
4. The agent multiplies only in actively growing tissues. It is not able to grow by itself but is carried away by the growth of dividing cells where multiplication in the living protoplasm is enormous.
5. Transport is 'through the phloem', upwards and downwards according to laws directing the movement of nutrients; in stems it is primarily vertical with little lateral spread.
6. The agent resembles living cells in that it is killed at 90 °C.
7. The agent may be dried in infected leaves (in a herbarium) and in filter paper soaked in infectious sap.
8. The agent may remain in dry soil during winter and infect plants from the soil; it can also be transferred in potting soil.
9. The agent retains infectivity after alcohol precipitation from sap and subsequent desiccation at 40 °C.

Beijerinck's conclusion, therefore, was that infection is not due to a microbe (a *contagium fixum*; Beijerinck 1898*a*), but to a non-corpuscular (that is, non-cellular) entity which he named *contagium vivum fluidum*. In the three versions of his paper the terms 'liquid state' and 'dissolved state' are used interchangeably (Beijerinck 1898*a*,*b*, 1900*a*). When, after his presentation at the Academy meeting of 26 November 1898, he was questioned by Hugo de Vries about the meaning of the adjective *vivum*, Beijerinck responded that he considered the ability to reproduce to be the major characteristic of life. Within the original undefined category of 'viruses' as a term to denote all sorts of venomous agents, of which *microbes* had become better defined thanks to Koch's Postulates, further to the lack of growth on artificial substrates filtration thus helped to define another subcategory: the *filterable viruses*. But it must be remembered that filterability was not Beijerinck's sole criterion for recognizing the agent as something new. The term *contagium vivum fluidum* is only used in the title of Beijerinck's paper and in the heading of two of its sections. Throughout his text, when referring to the agent, Beijerinck writes about the 'contagium' or more often the 'virus'.

There should be no misunderstanding: Beijerinck's virus is described as an entity fundamentally different from micro-organisms in, as we would now say, (i) its 'going systemic' in plants together with its metabolites; (ii) its multiplication in growing tissue; and (iii) its retention of infectivity in expressed sap after filtration and alcohol precipitation, and also after storage in desiccated leaves and dry soil. Beijerinck clearly indicates that the virus becomes part of the cell's metabolism: 'Without being able to grow independently, it is drawn into the growth of the dividing cells and here increased to a great degree without losing in any way its own individuality in the process'. We now know that there may also be virus multiplication in full-grown leaves at the site of virus introduction. This was recognized much later when inoculated leaves of some plant species were found to react by developing so-called local lesions, as with TMV in *Nicotiana glutinosa*, but at the time of Beijerinck's observations this phenomenon had not yet been noticed. Crucial is Beijerinck's awareness that the virus needs an actively metabolizing host. Beijerinck's later successor and biographer Kluyver (1940) clearly states that throughout the paper Beijerinck expresses a firm belief in the existence of an autonomous sub-microscopic (that is, subcellular) form of life. This is where an entirely new concept emerged. The prevailing theory that 'all viruses are microbes' was altered to 'a virus is not a microbe'. The word virus was getting an entirely new meaning, but was this going to revolutionize the study of infectious disease, or was it merely the threshold of change?

5. CHANGE OF PARADIGM OR PERSISTENCE OF THE OLD DOGMA?

Indeed, the crucial question now is: How radical was the conceptual change and did it influence other researchers and change the course of science, freeing pathology from the spell of the germ theory? Did it lead to a solution accepted by the profession, that is, to a real change in paradigm, a term coined in 1959 by Kuhn for scientific perception of reality (Hoyningen-Huene 1993). At that time, nobody yet knew what was actually going on at the subcellular level. Was Beijerinck's new concept more a matter of *belief* (the word perhaps unintentionally used by Kluyver (1940)) or *vision*, rather than a convincingly argued new theory? For an answer, we must now look at the reaction of Beijerinck's contemporaries.

(a) *An obsessive believer: Ivanovsky*

The year after Beijerinck's first reports in 1898, but most likely in reaction to the German version published in the *Centralblatt für Bakteriologie* (Beijerinck 1899*a*), the Russian biologist Dimitrii Ivanovsky retorted that he had priority in discovering the filterability of the agent of tobacco mosaic, and had done so as early as 1892 (Ivanovsky 1892, 1899). This was true and is acknowledged 'with pleasure' by Beijerinck in a short note in the same *Centralblatt* (Beijerinck 1899*b*) and as a postscript to the French version of his full paper (Beijerinck 1900*b*),

Figure 4. Postage stamp issued in Russia in 1964 on the occasion of the 100th birthday of Dimitrii Ivanovsky (1864–1920), claimed to be the 'founder of virology'.

both stating that at the time of his publication he was unaware of Ivanovsky's investigations. Should virology then be considered to have begun in 1892 when Ivanovsky published his results? This has often been claimed by the Russians (figure 4) and was also stated in a mini review in 1992 on 'One hundred years of virology' by Lustig & Levine (1992) and enlarged upon two years later by Levine *et al*. (1994) in their 'Foreword: 100 years of virology' in the then new *Encyclopedia of Virology*. 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 his exemplary 'pioneering spirit'.

Ivanovsky's (1892) short paper, read before the Academy of Sciences in St Petersburg in 1892, concentrated on claiming that Mayer's mosaic disease actually comprised two diseases, of which the mosaic component was thought to be identical to the pock disease studied earlier by Ivanovsky & Polovtzov (1890) in the Crimea. He then confirmed Mayer's finding of infectivity and finished by denouncing Mayer's conclusion that the agent loses infectivity by passage of the sap through two layers of filter paper. Without any detail he then, in one sentence, reports to have found 'that the sap of leaves attacked by the mosaic disease retains its infectious qualities even after filtration through Chamberland filter candles'. This finding is a landmark in the history of virology indeed, but what counts is how observations are interpreted and what they lead to. Let us look at the facts.

From the outset, Ivanovsky kept insisting that he was dealing with a microbe that might have passed through the pores of the bacteria-proof filter or might have produced a filterable toxin. In line with Koch, he obstinately kept looking for cultivable bacteria. When, in 1899, reacting to Beijerinck's report, he relates that by 1892 he himself had 'succeeded in evoking the disease by inoculation of a bacterial culture, which', he says, 'strengthened my hope that the entire problem will be solved *without such a bold hypothesis*' [author's emphasis] (Ivanovsky 1899). Beijerinck's concept is just denounced as a bold hypothesis. Kluyver, Beijerinck's successor in Delft, later wrote that 'anybody reading Ivanovsky's 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' (Kluyver 1940). Of crucial significance, in this respect, is Ivanovsky's dissertation on 'the mosaic disease of the tobacco plant', published in German in *Phytopathologische Zeitschrift* in 1903 while he was Professor of Botany in Warsaw, Poland (Ivanovsky 1903). The Americans (Lustig & Levine 1992) refer to Ivanovsky's interesting information on inclusion bodies produced by the virus also contained in that document, but they overlooked or ignored its most important section on '*the culture of the microbe of the mosaic disease*' [author's emphasis]. There, Ivanovsky categorically concludes 'that the contagium of the mosaic disease is able to multiply in the artificial media'. I have earlier inferred that 'this clearly demonstrates that Ivanovsky did not grasp the scope of his observations' (Bos 1995*a*,*b*). Ivanovsky's tenacity confirms the supremacy of the then current theory and 'shows the outcome when theory (Koch's Postulates) fossilizes into dogma' (Bos 1981).

(b) *The animal scene: Loeffler and Frosch*

Of great interest also are the observations on foot-and-mouth disease of cattle made by a commission headed by Loeffler and Frosch (Loeffler & Frosch 1898) at the Institute of Infectious Diseases in Berlin. Elaborate experiments were performed during 1897 and early 1898, at the time when Beijerinck was working on tobacco mosaic. The major conclusions drawn by the German investigators were as follows.

1. The disease can be artificially transferred in lymph from epidermal vesicles; bacteria that these sometimes contain do not reproduce the disease.
2. Lymph, filtered for isolation of an agent responsible for immunity developing soon after infection, is still infectious.
3. The infectious agent cannot be grown in artificial media.
4. The filtrate does not contain a toxin responsible for the disease but 'as yet undetectable disease agents so small that they were able to pass the filter pores retaining the smallest bacteria' including those of *Bacillus fluorescens* previously added as a control.
5. The infectious agent must be so small that it would indeed escape visible detection by microscopy (according to calculations by Professor Abbe, Jena, about the limit of resolution of the microscope used).
6. The agent is not soluble but 'corpuscular' because it is retained by a fine-pored Kitasato filter.

There is a remarkable parallel between Loeffler & Frosch's approach and conclusions and those of Beijerinck's, but those of the Germans did not lead them to refute the germ theory. At the end of their paper, they speculate that 'the agents of numerous other infectious diseases of man and animals, such as smallpox, cowpox, scarlet fever, measles, typhus, and rinderpest etc., so far sought in vain, belong to the group of these *minutest organisms*' [author's emphasis]. Their results were published in four reports with full documentation in the *Centralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* in the course of 1898 (viz, three by Loeffler & Frosch (1898); and the fourth by Loeffler (1898)). They appeared in German, which may be why they have achieved less fame than those of Beijerinck. The first report is dated 17 April 1897, so it may already have been presented in public in 1897, the year when Beijerinck started his investigations on tobacco mosaic. A summary of the German results was also published in 1897, viz in September that year (Loeffler & Frosch 1897), but this may not have attracted Beijerinck's attention since no mention is made of filtration experiments. Beijerinck must indeed have been familiar with the German findings. In a footnote added to the German version of his paper (Beijerinck 1898*b*) actual reference is made to the fourth report of the Germans (Loeffler 1898) in which he disagrees with 'the conclusion of Mr Loeffler as regards the corpuscular nature of the virus of the foot-and-mouth disease'.

(c) *Further confusion: Roux's 'so-called "invisible" microbes'*

Around the turn of the century there was increasing confusion about the criterion of size, and discussions arose about the limited importance of the associated criteria of filterability and visibility with the light microscope. An increasing number of diseases became known for which the agents could neither be seen nor cultivated in artificial media, suggesting their affinity with the new category of 'filterable viruses'. However, some have erroneously been drawn into the group due to inadequacy of the then prevailing but still incomplete definition of a virus. This now leads me to mention the plant diseases caused by agents recognized some 60 years later as 'pleuropneumonia-like organisms' (PPLO), later mycoplasma-like organisms (MLO), now the cell wall-less pleiomorphic prokaryotic *Mollicutes*. Until the 1960s they fitted the 'dark-age' definition of viruses (Bos 1981), and their relationships with plants and insect vectors were indistinguishable from those of viruses.

I have already alluded to the isolation of bacteria associated with yellow disease of hyacinth (Wakker 1883) and fire blight of apple and pear (Burrill 1886), and to the demonstration of their causal relationships with the diseases concerned, thanks to the newly emerging bacteriological techniques. Such techniques, however, soon proved unsuccessful for peach yellows and peach rosette, when they were studied in considerable detail by the American bacteriologist Erwin Smith. He was able to effect transmission of the diseases by budding and grafting, but was unable to isolate a bacterium (Smith 1888, 1894). Smith pointed to resemblance to graft-transmissible variegation of *Abutilon* and *Jasminum*, and Beijerinck (1898*a*,*b*) later ranked such variegations and peach yellows into one category of infectious diseases differing from tobacco mosaic only in that their agents are not sap-transmissible.

Of great interest are observations made during 1893 and 1894 by the Dutch botanist and geneticist Hugo de Vries on an 'epidemic of virescences' (de Vries 1896) while carefully examining various plant species in his garden for the occurrence of mutants. The epidemic observed by de Vries, that is virescence (greening) and phyllody of floral organs, must have been symptomatologically identical to those of aster yellows, described in the USA much later by Kunkel (1926) and related aetiologically to that disease and to peach yellows (Bos 1957, 1966). After considerable hesitation to publish his results because 'repeatedly I have tried, macroscopically and microscopically, to find parasites, but so far in vain', de Vries finally gives in and writes that he is 'convinced of the infectious nature of the disease and of its spread by flying insects' and he reports his data 'in the hope that others may later be more successful in finding the parasite'. It was not until the 1920s and early 1930s that L. O. Kunkel at the Boyce Thompson Institute found aster yellows (Kunkel 1924, 1926) and peach yellows (Kunkel 1933) to be transmissible by leafhoppers. This warranted the grouping of their agents with viruses according to the then prevailing criteria. Thus, at the time when Beijerinck announced his new concept, there was an increasing number of odd diseases that seemed to comply with Beijerinck's outlook. The observations by de Vries may well have been another incentive to the detailed investigations started by Beijerinck in 1897.

Highly significant now, because typical of the reigning confusion, and of great influence on the then current opinion, is a review by Roux (1903) published by the prestigious Institut Pasteur in Paris on maladies caused by the filterable and invisible disease agents, perhaps the first for viruses. It bears the suggestive title 'Sur les microbes dits "invisible"' ('On the so-called "invisible" microbes') and, among others, includes the agents of Beijerinck's tobacco mosaic as well as of bovine pleuropneumonia. This is where the confusion began to escalate. The cattle disease had been investigated by Roux and others (Nocard *et al*. 1898) in 1898, when Loeffler & Frosch and Beijerinck reported their findings. The disease first seemed also to defy Koch's Postulates, but its agent could after many fruitless efforts be cultivated under very specific conditions, and be made visible in the light microscope at high magnification, although 'so small that their form is difficult to define'. When, after Nocard's presentation of the paper during a conference in Madrid, Loeffler asked whether filtering experiments had been done, the answer was negative. However, the year after, Nocard found that it did pass Berkefeld and Chamberland filters at high dilution of the agent-containing lymph (Loeffler 1911). This then seemed to justify inclusion of the agent in the new category of invisible agents and to substantiate Loeffler & Frosch's idea of a small microorganism associated with foot-and-mouth disease. Roux's (1903) paper, also listing Beijerinck's tobacco mosaic contagium, concludes that 'One cannot say that the microbe of pleuropneumonia is invisible, it is at the limit of visibility, it forms *a transition between the ordinary bacteria and those which the microscope is incapable of showing*' [emphasis added]. The agent of pleuropneumonia was regarded as the smallest microscopically visible member of a continuous chain of minute organisms that extended from those of microscopically visible dimensions to others which were beyond the reach of the light microscope. The existence of submicroscopic organisms, so far only a matter of speculation, was then viewed as a virtual certainty (Smith Hughes 1977). Pasteur's 'all viruses are microbes' continued to echo.

Filterability and invisibility were thus losing weight as criteria for distinction between cellular and non-cellular agents. We now know that the result of filtration depends on pore size. There are filters that retain large viruses, and some viruses may be lost on bacteriological filters because of electrostatic attraction. *Mollicutes* may indeed pass bacteria-retaining filters. Success in cultivating the 'invisible' agents on cell-free media was also considered a relative criterion and a mere matter of proper choice of medium. Roux, therefore, considered Beijerinck's *contagium vivum fluidum* idea 'très originale', but he could not exclude the existence of a very small microbe possessing spores. Proof of the actual involvement of a mycoplasma-like organism in pleropneumonia had to wait until 1962 (Chanock *et al*. 1962) and of 'pleuropneumonia-like organisms' in aster yellows-like plant diseases for another five years (Doi *et al*. 1967).

Seven years after Roux's review, his ideas were further supported by a review 'Ueber filtrierbares Virus' by Loeffler (1911). He refers to Beijerinck's paper on the *contagium vivum fluidum* by briefly concluding that the author did not provide 'further proof for this view'.

Thus, microbiological dogma continued to dominate the scene, particularly in the medical field. To this day some medical and veterinary virologists may still talk in terms of organisms when dealing with viruses. Viruses are more than merely invisible, filterable and uncultivable things. A defining feature is their very special relationship with the host, as first voiced by Beijerinck but not grasped by the zoologists, that is, their becoming part of the host's metabolism. At the turn of the century, the time had not yet come to appreciate Beijerinck's conclusions, let alone to verify his hypothesis. But evidence against the universality of the germ theory accrued and there were people other than Beijerinck also of differing opinion.

6. ANOTHER RENEGADE: BAUR

Various mosaic-like variegations, such as that of *Abutilon striatum*, often passing under a name of its own, *Abutilon thompsoni* as if it is a genetic variety (figure 5), had long been known to be infectious. In 1869, Lemoine (1869) in France, Morren (1869) in Belgium, had proved transmissibility of *Abutilon* mosaic by grafting. The phytopathological classic 'On the etiology of infectious variegation' by the German geneticist Erwin Baur (1904) is revealing. Baur describes the symptoms, their ornamental use and propagation by cuttings, transmission by grafting, lack of seed and sap transmissibility, and possible spontaneous spread in the tropics. Historically, most interesting, however, is Baur's discussion on the aetiology of the phenomenon in view of the absence of 'any kind of parasitic foreign organisms'. Baur's observations led him straightforwardly to conclude that the agent '*cannot be a living organism*' but must be 'a non-organized, let us say *a pure chemical substance*, . . . able to assimilate foreign substances, in order to rebuild itself from them' although 'such a substance is yet unknown to us' [emphasis added]. He later stresses that, consequently, 'the *dogma* that an infectious disease without a living (organized) cause is inconceivable, a dogma which dominates the whole field of the pathology of infectious diseases, must be wrong' [emphasis added]. He then also dwells upon the fact that 'one must not by any means conclude that a virus must grow "actively", as an organism grows'. It would be conceivable, for example, that the '*the virus might function as a product of metabolism of the diseased plant*' [emphasis added]. He ends by referring to a whole series of infectious diseases, above all, the mosaic disease of tobacco, without specifically mentioning Beijerinck, 'where all our knowledge to date contradicts organisms as a cause . . . For a further insight into the aetiology of these diseases the old dogma of the unconditionally parasitic nature of all infectious diseases seems to me only an obstruction'.

Figure 5. Symptoms of graft-transmissible variegation of *Abutilon striatum* now known to be caused by *Abutilon* mosaic virus, a begomovirus. (Photograph: IPO-DLO, Wageningen.)

7. FINAL BREAKTHROUGH: MOLECULAR BIOLOGY

While Beijerinck stressed the non-organized, non-cellular but still *living* nature of the new category of disease agents, Baur seems the first to emphasize their mere *chemical*, that is, *non-living* quality. The true nature of viruses thus remained a matter of speculation for three more decades. Viruses were increasingly compared with enzymes, as already done by Mayer (1882), or with genes (e.g. Duggar & Armstrong 1923). It is of interest here to note that Beijerinck, originally trained in chemistry and with a bent towards physiology, had already ascribed the material base of heredity to enzymes (Beijerinck 1900*b*,

Figure 6. Bottle with part of the suspension containing paracrystals of TMV, isolated in 1935. It was proudly shown by Stanley during a TV programme in 1960, 35 years after isolation and then still capable of causing disease in tobacco plants. (After Stanley & Valens 1962.)

1917). But in the no man's land before molecular biology got off the ground, nobody knew the nature of either enzymes or genes, nor the relationships between them.

Viruses could, for a long time, only be studied for their transmissibility from plant to plant and for their reaction on plants, i.e. for their rather variable *biological properties*. But, particularly during the 1920s, when an increasing number of virus diseases, then especially referred to as *mosaic diseases*, were described and distinguished by differences in symptoms, hosts, and ways of natural and artificial transmission, the urge for information on the *intrinsic properties* of their agents increased. One attempt was James Johnson's (1927) description of the so-called 'physico-chemical virus properties', a challenging but now misleading term for nothing but the persistence of the infectious agents in expressed sap on dilution, heating, chemical treatment and storage.

Real change only commenced in 1935 when the chemist Wendel M. Stanley was appointed by L. O. Kunkel in the then newly established plant pathology laboratory of the Princeton branch of the Rockefeller Institute to work on a more chemical approach to studying the still enigmatic viruses. Next door at the Institute, Northrop had just successfully isolated and crystallized trypsin, pepsin and other enzymes. In his footsteps, Stanley (1935) soon succeeded in isolating 'a *crystalline protein* possessing the properties of tobacco mosaic virus' [emphasis added] (figure 6). This later qualified him, together with Northrop and Sumner, for the 1946 Nobel Prize for Chemistry. Although erroneously describing TMV as an autocatalytic enzyme, the American achievement, together with the characterization of the virus as a *protein–nucleic acid complex* by Bawden & Pirie (1937) in Great Britain soon thereafter, was *the* breakthrough in our understanding of viruses as truly physicochemical entities and the later revelation of their role as genetic entities. This is how *molecular biology* got off the ground, the new field of science which, in turn, provided the methodology and tools for further physico-chemical characterization of TMV (for an interesting review, see Kay (1986)) and other viruses up to the amazing detail discussed in this issue. It goes beyond saying here how much electron microscopy, applied to TMV, the first virus of which the particles were ever seen, in 1939 (Kausche *et al*. 1939), has helped to eliminate much of the mystery shrouding the hitherto invisible disease agents that had for long remained 'beyond the microscope' as voiced by Kenneth Smith in 1948 (Smith 1957). It still took until 1967 for electron microscopy also to solve the riddle of Roux's so-called '"invisible" microbes', that is, to prove that the agent of bovine pleuropneumonia and the mycoplasma-like agents of plants really differ from viruses (Doi *et al*. 1967), so that the definition of viruses could be refined further.

These developments are testimony to how close Beijerinck had been to the mark when precipitating the soluble infectious principle contained in sap from mosaic-diseased tobacco plants, in earmarking it as filterable and invisible, but more so in perceiving it as a new type of disease agent, unique in its intimate involvement in the metabolism of the host, being 'drawn into its growth ... and here increased ... without losing in any way its own individuality ...'.

Since 1898, some 40 years were needed for science to develop the methodology and technology required for characterization of viruses. Several more years had to elapse to allow final description of their molecular nature as non-cellular small packages of host–alien genetic information, supreme representatives of obligate parasitism lacking any physiological machinery of their own; viruses live 'a borrowed life' (Laidlaw 1938). Finally, the long-lasting controversy over whether viruses are organized living beings or 'non-organized' chemicals, animate or inanimate, could be reconciled. Viruses are at 'Life's fringes' (in Dutch also to be read in retrograde order: 's' Levens nevels') as already brilliantly discussed by Beijerinck's successor Kluyver in a conference address in 1937 (Kluyver 1937). The study of viruses had much to say about the *nature of life* (Stanley & Valens 1961). In their design and function, viruses really are *at the threshold of life* (Fraenkel-Conrat 1962) and thus of utmost interest to biologists.

8. BEIJERINCK'S LEGACY

Turning to consider Beijerinck's legacy to virology, in terms of spin-off and follow-up, we must now conclude that there is no such direct legacy. Beijerinck's classical 1898*a* paper, postulating the existence of an entirely new type of pathogen, though considered to mark the beginning of the change of paradigm with respect to the nature

Figure 7. Collected papers by M. W. Beijerinck, published in 1921 on the occasion of his 70th birthday (and retirement). The sixth volume was published in 1940, and contains his publications that appeared after 1920, together with indexes to all volumes and a biography by Van Iterson, den Dooren de Jong and Kluyver discussing his life and his work.

Figure 8. Cartoon of Professor Adolf Mayer about 1900 by Louis Raemaekers, who was then teacher of hand-drawing at the Agricultural College, later well known for his political cartoons during World War I which were published in for example, the *Daily Mail*, for which he was awarded a doctor's degree honoris causa by the University of Glasgow, UK, in 1922, and in *The Times*. Mayer is depicted as Goethe's Dr Faust with Mephistopheles as the symbol of evil in the background. It testifies to awareness of Mayer's (and Beijerinck's) involvement in phenomena at the threshold of life and reservations with respect to the possible outcome of modern Cartesian science. The cartoon also symbolizes the embryonic beginning of virology. (Reproduced with permission of the Historical Collection, Agricultural University, Wageningen.)

of 'viruses', did not lead to revolutionary change in science. At the time no new school of thought and research resulted. Methodologies and tools for pursuing studies to verify Beijerinck's visionary views just were not there. That must have been why immediately after 1898, Beijerinck returned to the discipline to which he had been appointed and which he was equipped to study: bacteriology. This is also the domain in which he became astonishingly productive, as demonstrated by the five monumental volumes of 'Collected papers by M. W. Beijerinck' (figure 7), published by friends and admirers in 1921 on the occasion of his retirement at the age of 70 (see also Van Iterson *et al*. 1940). For that, he rightly achieved world fame, including memberships of learned foreign societies such as the Royal Botanical Society of Edinburgh (in 1906) and the Royal Society of London (in 1926: Bulloch 1932).

In 1931, after ten years of retirement living as a bachelor with two sisters in the quiet town of Gorssel, province of Gelderland—where he returned to botany, for example studying the mathematics of phyllotaxis—Beijerinck passed away at the age of nearly 80. He had not lived long enough to witness the full discovery and final characterization of his tobacco mosaic contagium that occurred later during the 1930s. In this respect, there is a striking similarity between Beijerinck's fate in virology and that of Mendel in genetics. Some 35 years also were needed until Hugo de Vries, Correns and Chermak rediscovered the 'Mendel laws' in 1900, soon after ascribed to the action of the *genes* christened by Morgan. In fact, Beijerinck was 'the Mendel' of virology but he had a clear notion already of the close association between virus and host metabolism.

So, there is no direct legacy of Beijerinck's to virology. What must be remembered is his critical observation of nature and radical way of thinking, unbiased by prevailing theory, in an endeavour to come to grips with an obviously novel group of pathogens. This is where Beijerinck towered above others such as Ivanovsky, Loeffler & Frosch, and Roux. One lesson is that our images of reality should not fossilize into *graven* (that is, carven) *images*, unalterable as if cast in stone. The birth of virology fascinatingly exemplifies the struggle of science in search of truth in order to grasp reality in concepts and images, and how this is a matter of trial and error. With respect to viruses, outlook did not suddenly change.

The actual change of paradigm, that is the conceptual conversion and general acceptance by the profession, turned out to be a time-consuming process. However, there is no doubt that *conceptually* virology was conceived in 1898 when Beijerinck's classical paper (Beijerinck, 1898*a*,*b*) was published. The development of the new discipline remained embryonic (Bos 1995*b*) until its actual birth, in 1935, when viruses became subject to isolation and study *in vitro*.

No matter how original, Beijerinck's achievements, like ours, are influenced by or build on those of many others. Although he did not explicitly say so when referring to Mayer's (1886) report (Beijerinck 1898*a*), Beijerinck must have known about Mayer's (1882) ideas about the possibly enzyme-like nature of the agent of tobacco mosaic. Therefore, *de facto*, the roots of plant virology go back to Mayer's earlier work, especially that published in 1882 (Mayer 1882). That is why in Wageningen we celebrated a centennial of virology 16 years ago (see de Bokx *et al.* 1982). In the album presented when Mayer retired from office in 1904, Beijerinck's photograph occupies a place of honour on the front page. Mayer's colleagues at Wageningen must have known about Beijerinck's far-reaching vision and have realized how close both had already been to the mark in suggesting the nature of viruses at the threshold of life.

9. CONSEQUENCES BEYOND VIROLOGY

Early appreciation in Wageningen of Mayer and Beijerinck's exceptional idea and its consequences is testified to by the remarkable cartoon (figure 8), made by one of their Wageningen colleagues, Raemaeker's, at the turn of the century, a teacher of hand-drawing at the school. It was made in 1900 (de Ranitz 1989) soon after Beijerinck's (1898*a*,*b*, 1899*a*, 1900*a*) publications, by which it must have been inspired. The cartoon depicts Mayer as a chemist from the school of Liebig, but also as Goethe's Dr Faust approaching the threshold of life with Mephistopheles, the symbol of temptation and evil, in the background. It strikingly represents the old dilemma between *vitalism* (awareness of something special in life, be it a vital factor or just natural complexity never tangible in its entirety) and *mechanicism* (the Cartesian, analytical, reductionist, or physico-chemical approach, as if life were nothing but a machine). In agriculture, technological development commenced with Liebig's agricultural chemistry (as also represented and introduced into the Netherlands by Mayer). Largely stimulated by the study of viruses like TMV, it was later followed by molecular biology, and biotechnology, reaching the threshold of life, was the final outcome. Raemaekers' cartoon may be more topical than ever, reaching far beyond virology.

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