

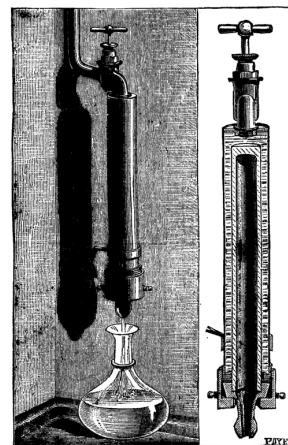
# Viral models in virology

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Virology is a relatively new science. It's always very difficult to give a precise date of birth for each science. Can we speak of "virology" if viruses are "discovered" but not distinguished from, for instance, bacteria? Speaking of virology requires to have a well-defined concept of virus: how was this concept built? I will show that virology "came to exist as a well-defined science" inside a long period that goes from the end of the nineteenth century until the middle of the twentieth century [1892- 1960s]. I particularly focus on the shorter period going from the 1930s to the 1950s. Two important points will be underlined here:

- The construction of virology relied on the use of specific viruses taken as "*models*". In what sense these viruses were "models"? How can we explain the choice of these viruses, rather than other viruses?
- The use of some very *specific experimental sets* was a decisive aspect of the establishment of a stable concept of virus, shared by the whole scientific community of biologists and physicists. This concept, before being "theoretical", was experimentally determined.



**Figure 1 Chamberland's candle**

## Viral models

Until the 1890s, "virus" is a general name for all types of infectious particles. Thanks

to the Chamberland's candle, acting as a filter retaining the biggest particles, infectious particles ("viruses") can be distinguished between spores, toxins and bacteria. For instance, bacteria are captured by the filter, whereas toxins go through it. But what if a particle goes through the filter, without behaving like a toxin? What are these "invisible microbes", that may behave "like" bacteria, but being much smaller since bacteria could normally be observed through an optical microscope? After numerous filtrations, two scientists made two different hypotheses on the "nature" of the "ultrafilterable viruses": while Dmitri Ivanovsky assumed that these particles may be toxins or little bacteria, Martinus Beijerinck claimed that they must be "new", unknown particles, which he called "contagium vivum fluidum."

Between the 1890s and the 1920s, four of these "ultrafilterable" viruses were discovered:

- The Tobacco Mosaic Virus, plant virus, first to be discovered, was the subject of the numerous experiments made by Ivanosky and Beijerinck in the 1890s.
- The foot-and-mouth, an animal virus, was discovered by Löffler and Frosch in 1897.
- Bacteriophages, viruses of bacteria, were "discovered twice": by Frederic Twort in 1915, and by Félix d'Hérelle in 1917.
- Yellow fever virus, human and animal virus, was isolated in 1920 from apes, human beings and mosquitoes.

Among these viruses, one may think that the model virus would be the human virus, since human health appeared (and appears) to be of great importance. And yet, Tobacco mosaic viruses and bacteriophages became much more central as models than the two others, including yellow fever virus. How can we explain both this choice and the ability to extrapolate from these viruses to other viruses? What were the limits and the difficulties for extrapolation?

### **"Model system"**

A model in virology may be either a specific object or entity (a virus) whose properties may be, under certain conditions, extrapolated to other objects. But it also can be a given experimental and theoretical set used to study some viruses: in this case,

the “model” is more the “*way of studying*” than the “*what is studied*.” This distinction between two types of model is based on the analysis made by Angela Creager, in her book *The Life of a virus: Tobacco mosaic virus as an experimental model, 1930-1965* (2002). Analyzing the use of the expression “model system” in the scientific laboratories where TMV was studied (especially Wendell Stanley’s laboratory), she noted that the expression was either used to designate a “standard prototype” or a “research exemplar.” In the first sense, TMV is studied as a *representative* virus, “with the expectation that knowledge gleaned from TMV could be generalized (provisionally) to other viruses.” (p. 5) The model system is here the biological object and its properties. But Angela Creager insists on the fact that this is not the most useful (and used) sense of “model system” in the lab. It seems to be much more important to extrapolate not the “biological properties” of the virus, but the experimental and theoretical set built on it. What really matters, is not to find “analogous properties” in other viruses, but to determine how far our theoretical and experimental tools *resist* when confronted to new cases. TMV was essential as a model system because it was the centre of an experimental and theoretical set.

## **Tobacco Mosaic Virus (TMV) in Stanley’s lab**

The use of the electron microscope at the end of the 1930s was certainly a crucial step in understanding viruses: the invisible microbes became visible. But what is really seen through a microscope? How may the images be interpreted? For all these reasons, it seems that the electron microscope, *alone*, couldn’t be crucial. Indeed, it was preceded, a few years before, by another experimental disposal that largely constrained the understanding of TMV: crystallization *via* precipitation. In 1935, Stanley’s lab isolated TMV in the form of a crystallizable material: if the virus may be crystallized, this could mean that this biological entity is a protein. This was the first hypothesis made by Stanley: viruses are proteins, and more precisely *autocatalytic* proteins, being able of “reproducing themselves.” This was extremely important for two reasons: first, this provided an experimental (crystallization) and theoretical (biochemistry) set that

could be extrapolated to other viruses; second, the concept of virus turned from a “little bacteria” or from a “contagium vivum fluidum” to a “protein”. At this point, viruses were supposed to be “chemicals”, “reproducing” macromolecules similar to proteins, despite their unusually high molecular weight.

And yet, this hypothesis was already falsified the next year thanks to another decisive experiment. The analytical centrifuges, developed in the 1920s and 1930s by physical chemists, were an important tool to determine the size and shape of molecules: spinning at high speeds in the analytical centrifuges, molecules came to sediment in a solution; the way they sediment gave indications about their size and shape. In 1936, analytical centrifuges convinced biologists that viruses weren’t “only” proteins. They proved to be “nucleoproteins” (made of a nucleic acid and a protein). But how could one determine the respective roles of the nucleic acid and the protein in a virus’ infectivity and replication?

As Stanley reported in his “Penrose Memorial Lecture” (1957), the “Fraenkel-Conrat” experiment realized in 1955 was essential: “It was reported by Fraenkel and Conrat and also shortly thereafter by Gierer and Schramm in Germany that special treatment of tobacco mosaic virus yielded a nucleic acid preparation possessing virus activity. It would now appear necessary to recognize that a nucleic acid structure of around 300,000 molecular weight can possess coded within its 1000 or so nucleotides not only all of the information that is necessary to bring about in the host cell the production of more of this same nucleic acid, but also apparently the *de novo* synthesis of its own characteristic and highly specific protein with which it eventually coats itself.”

In the mid 1950s, viruses were defined as nucleoproteins whose replication and infection are determined by the nucleic acid alone. The experimental and theoretical set built around TMV (the “model system”), articulating theoretical biochemistry and physico-chemical instruments and experiments (crystallization, centrifugation and preparation of an active nucleic acid solution), was used in a great number of laboratories working on viruses. But can a “model system” be easily extrapolated?

## **Extrapolating from TMV and the problematic diversity of viruses**

The diversity of viruses may limit the usefulness of extrapolating TMV's model system. Indeed, TMV is a plant virus; and many biologists were convinced that the different domains (plant, animal, human) had to be conceptually separated; so do their viruses. But Stanley's lab showed that (at least some) animal and human viruses could also be obtained in a crystalline form: poliomyelitis virus was crystallized in 1955. The assumption that different domains exist and should be separated was no longer an obstacle against the extrapolation from TMV.

But the diversity of viruses is problematic in another sense. As Stanley noted (1957, *ibid.*): "Hundreds of viruses are known and more are being discovered every month; yet only a dozen or so have been obtained in purified form." Viruses present namely various degrees of morphological differentiation: most of them appeared to be more complex than "simple" nucleoproteins, since they possess a lipid envelope. Is it necessary to give up any kind of extrapolation when faced with these viruses? Is the model definitively limited? It was certainly essential to develop specific experimental means to study the more complex viruses, but one would be wrong to say that "nothing" was extrapolated from TMV.

- Considered as a "research exemplar", TMV's model system was essential in revealing the existence of a viral diversity different from the expected one. Viral diversity may not be a question of "domain", but a question of morphological differentiation. Moreover, Stanley used his centrifuge-based method to develop a new kind of influenza vaccine. "Thus TMV served as a model for applied research on influenza virus." (Creager 2002, p.6)
- Considered as a "standard prototype", TMV was crucial in giving a unified concept of virus. Viruses were understood as macromolecules, genetic units protected by a protein coat, parasites that depend on their hosts for metabolism and reproduction. This was the "concept" of viruses in the 1950s.

Even if the model system built on TMV was limited, it certainly contributed to the construction of research programs (morphological differentiation of viruses; vaccines), guiding the construction of both medical and biological virology.

## **Choice of a model: Historical precedence and biological robustness**

One last question remains: why TMV? As Angela Creager says, “researchers constructed general knowledge about viruses based on a few that, by reason of historical precedence or biological robustness, were intensively studied as representatives of the rest.” TMV, because it was the first virus to be discovered, was immediately designated as a potential model. And yet I claim that historical precedence could never be sufficient. It’s true that the “history” of the virus is essential: the more knowledge is accumulated on a virus, the more easy it is to handle in the lab and to formulate precise hypotheses. But the choice of a model is also determined by other reasons, like economical aspects (the agriculture of Tobacco) and experimental reasons: a model must be easy to obtain (low cost, high reproduction level), and easy to handle (isolation and identification of the components, dangerousness towards human beings – and towards the scientists who manipulate the virus). A human virus is supposed to be much more dangerous than a plant virus (which sounds quite logical, even if it’s not obvious). The choice of a model virus is strongly related to the kind of host this virus could infect.

These economical and experimental reasons may explain the choice of TMV and constitute its “biological robustness”. Moreover, some similar reasons enlighten the fact that TMV was competing with another “research exemplar”: bacteriophage.

## **Bacteriophages as models**

Bacteriophages were discovered by Frederick Twort in 1915 and by Félix d’Hérelle in 1917. In 1926, D’Hérelle described how to handle T-bacteriophages (Type-bacteriophages) in a microbiological laboratory. Intensive work of D’Hérelle’s lab on (T-) Bacteriophages turned this multiple biological object into a model for viral infections. But D’Hérelle was not only interested in the biological description of T-bacteriophages: he saw them as potential agents against dangerous bacteria.

In the 1930s, the research program of Emory Ellis was completely different, as he chose T-bacteriophages as model: Ellis was studying cancers, and more precisely cancers

caused by viruses. Why did he choose T-bacteriophages? It sounds surprising to study cancers caused by viruses with the help of viruses unable to cause any cancer because their host can't have a cancer (a bacteria can't have a cancer)! And yet, the problem of Ellis was a particular aspect of the viral infection: the "adsorption step". How could a virus enter a cell? And for this task, a bacterial virus, entering the bacteria, may be of good help. But this still doesn't completely explain the choice of T-bacteriophages. As explained by David Rowland in his book *Microbial models of molecular biology* (2003), Ellis chose them, *and not TMV*, for many reasons. Experiments with TMV were too costly and time-consuming; T-bacteriophages were cheaper and their replication rate (as the one of their host) was higher. Bacteriophages appeared to Ellis to be better viral models. Two other reasons made Ellis select the T-bacteriophages, among the various kind of bacteriophages: the important work of D'Hérelle (historical precedence) and the ability to "see" the effects of the virus on a population of bacteria with the naked eye (experimental reason).

T-Bacteriophages were good laboratory models, competing with TMV. But in what sense did they contribute to the constitution of virology and to the concept of virus?

### **Ellis & Delbrück: Physical virology**

Phages (short term for bacteriophages) became central in virology because a particular type of research structure was built around them. In 1937, Max Delbrück, atom physicist, joined Emory Ellis. The two scientists were searching two different things: Ellis was still interested in the adsorption step; Delbrück was trying to quantify mutation. But working together, they imported physical approaches in virology. The different steps of the virus life cycle (adsorption, replication of the nucleic acid, production of protein coats, assembly of acid nucleic and protein coats, cell/bacteria destruction) were not only characterized: they were quantified. For instance, they determined that the phage progeny (number of phages produced) per bacterium is quite reproducible. This "physical" turn of virology was reinforced by the creation of the Phage Group.

## **The Phage Group: Interdisciplinary virology**

Delbrück, together with Salvador Luria, constructed an interdisciplinary group around the phages. Biochemists, physicists, chemists, mathematicians, biologists were working on the same biological object, articulating different methods and approaches. The “model system” here has to be distinguished from the one built on TMV: while the scientists working on TMV were mostly biochemists, phages were studied by different scientists whose approaches were articulated (and sometimes competing); nevertheless the physical approach was often dominating the other approaches.

This interdisciplinary structure gave birth to a real “community”, sharing methods, communication means, and standardized objects. For people working in the “Phage Group”, bacteriophages were classified (in “types” 1, 2, 3...) and standardized, reducing the role of hazard; finally only the phages T 1 to T 7 came to be used. A letter was used to inform each member of the community of the results obtained by other members (the *Phage Information Service*).

The Phage Group had a great impact in moving the phages from a “convenient” model to a model system able to transform the way virology was made. However, this intensive work on phages revealed their unexpected complexity and lead to modify the research community.

## **Biological turn of the “phage virology”**

The first electron micrographs of phages may be considered as the starting point of the “biological turn” of the phage virology. The electron microscope indicated an unexpected morphological complexity of phages, and lead to the discovery of “ghosts” of the phages at the surface of bacteria, while the infection was processing. What are those ghosts? How can a bacteria be infected if the virus remain outside? This observation was probably the first step to a fruitful hypothesis (by Roger Herriotth): maybe only the nucleic acid enters the bacteria; and the “ghost” may be the protein coat, that doesn’t enter. But to resolve this problem, phage virology needed more electron microscopists (like Thomas F. Anderson) and biochemists (like, for instance, Alfred



Hershey). Physicists and mathematicians were no more at the center stage of virology, replaced by biologists and biochemists.

This biological turn of virology led to important discoveries and experiments. In 1946, Hershey described the phenomenon known as “recombination” (the ability of different viruses, present in a same bacteria, to exchange genetic material). In 1952, Hershey and Martha Chase constructed an experiment that could prove or refute the hypothesis of Roger Herriotth. They used radioactive sulfur to label the protein coat; and radioactive phosphorus to label DNA. As summarized by Rowland Davis, “labeled sulfur had been largely removed from the bacteria, but the labeled phosphate largely remained with the bacteria until lysis. The demonstration that [...], suggested that DNA, not protein, was the substance of phage genetic material.” Three years before the Fraenkel-Conrat experiment in Stanley’s lab, this crucial experiment helped scientists to understand the crucial role of the nucleic acid.

## **Two competing exemplars**

These two models and the evolution of the theoretical and experimental systems built around them, gave birth to “virology” and an unified concept of virus. These two models converge in the 1950s and are, finally, complementary, but they were competing. While TMV’s model system insisted on the usefulness of biochemistry to study viruses from the beginning, phage virology was first a physical virology. While phage virology came to a high degree of standardization, TMV virology underlined the importance of extrapolation and the difficulties appearing in this process. One difference between these two models is often quoted: phages are “at the origin of molecular biology”, whereas TMV isn’t. These viral models would, according to this point of view, be equally necessary to understand the construction of virology, but not to understand the construction of molecular biology. However, this claim requires to be able to clearly distinguish between molecular biology and biochemistry, which is absolutely not an easy task.

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