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Bacteriophage: The contexts in which it was discovered

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1. Introduction

In April 2017, the Pasteur Institute in Paris organized a 'Centennial Celebration of Bacteriophage Research' to commemorate the discovery of the bacteriophage by Félix d'Hérelle in 1917. Although the expression chosen –'bacteriophage research' and not 'bacteriophage discovery' – emphasized the uncontroversial role that d'Hérelle played in putting the bacteriophage in the limelight, this choice might also be viewed as latter-day support of d'Hérelle's claim of priority in the discovery of the now universally named Twort-d'Hérelle phenomenon.

This famous controversy has already been extensively studied (Duckworth 1976; Summers 1999; Prangishvili 2007), and my intention is not to add any new 'revolutionary' information. Rather, I simply want to emphasize the different contexts in which the observations of Frederick Twort and d'Hérelle were made.

Context is highly important in historical studies, but this word may have different meanings. It may be the place (institution) in which a discovery was made, and the precise circumstances that led to this discovery. The context may have a more conceptual content, requiring the description of the research programmes in which the discovery was made as well as of the theories and models that permitted the interpretation of the experimental observations. This conceptual context is often the consequence of a deep history that far preceded the discovery under study.

I will show that both types of contexts are important in explaining the controversy, but also the characteristics of the bacteriophage that were immediately attributed to it by d'Hérelle. Some aspects of these contexts have not been sufficiently taken into account so far. I will first describe the physical context in which the two discoveries were made. Then, I will successively examine the research programmes of Twort and of d'Hérelle, and show how different they were: the discovery was peripheral to Twort's project, whereas it was central to d'Hérelle's. Finally, I will explain some of the characteristics attributed by d'Hérelle to the bacteriophage by the long traditions of research in which his work was rooted.

A last word: I will not examine the allegation by d'Hérelle (and others) that Twort did not observe the bacteriophage, the allegations of Twort's supporters that d'Hérelle knew of Twort's article before publishing his own, or the late claims of d'Hérelle that he had observed the bacteriophage years before Twort, but not published his observations. Twort's publication in *The Lancet* is sufficiently precise to leave no doubt that he was the first to describe bacteriophage effects. Nor will I revisit the long controversy with Jules Bordet on the endogenous or exogenous nature of the phage, which has also been extensively studied by historians (Van Helvoort 1994; Summers 1999).

2. The physical context of the discovery

Twort worked at the Brown Animal Sanatory Institution in London. The year in which he published his observations on what d'Hérelle would later call 'the bacteriophage', Twort was sent to Salonika, where he was unable to pursue his research. He returned to his laboratory only in 1919, with the feeling that it was too late to contribute to the new field of research. D'Hérelle worked at the Pasteur Institute in Paris.

The war, and the reorientation of the institute to support it (Perrot and Schwartz 2016), never prevented research from being pursued. The opposite was true: the Pasteur Institute was sixty miles from the battle front, and less distant from the troops among whom epidemics regularly emerged and required the presence of Pasteurians to be characterized. Research at the institute was stimulated more than hampered by the war!

3. Twort's research programme

Also different were the research programmes during which Twort and d'Hérelle made their respective observations.

Whereas d'Hérelle's article looks simple (d'Hérelle 1917) and his conclusions are straightforward, even for a 21st century biologist, Twort's article is difficult to read (Twort 1915) and his conclusions are less firmly stated. The reason is that Twort's research programme was not only foreign to the observation of the bacteriophage, but also meaningless in the framework of current knowledge. In his publication, Twort describes the efforts that he deployed to cultivate the vaccinia virus by using numerous culture media. He observed that some of his cultures were contaminated by bacteria, and noticed the appearance of glassy areas in the bacterial culture. He interpreted them as the result of bacterial lysis. He showed that the lytic substance was present in a filtered extract, and active at a very low dose. He proposed different interpretations of the phenomenon, among which the existence of an exogenous lytic agent able to reproduce within bacteria, and the bacterial secretion of a lytic self-reproducible substance.

Trying to cultivate vaccinia virus and paying attention to contaminating bacteria in the culture are erratic behaviours for a contemporary microbiologist. But the scientific context was totally different for Twort. The reasons why viruses and some bacteria could not be grown in media traditionally used for microorganisms were not obvious in the 1910s. Twort and many other bacteriologists thought that a substance necessary for their growth was missing from these culture media. Twort himself had brilliantly confirmed this hypothesis three year earlier in the case of Johne's bacillus, which causes a chronic intestinal infection of cattle: he had obtained bacterial growth through the addition to the culture medium of dead tubercle *bacilli*. Twort's reason for being interested in bacterial contamination was that he thought that the contaminants could eventually provide the missing substance, and therefore help to identify it. The search for these substances permitting the growth of microorganisms was considered fundamental for the production of vaccines. In particular, it was hoped that non-pathogenic forms of the microorganisms responsible for diseases could be cultured. These non-pathogenic forms were supposed to exist in the environment and were considered as ideal material for the production of vaccines. Twort was searching around the time when vitamins were being described and shown to be essential for the life of organisms, including humans. The discovery of the bacteriophage was totally peripheral to the main research Twort had been pursuing until then! The existence of a 'dissolving substance', as he initially called the bacteriophage, was a threat to the projects that he had developed in previous years.

4. D'Hérelle's research programme

The opposite was true for d'Hérelle. He was not looking for something similar to the bacteriophage, but since 1909 he had devoted most of his time to the use of microbes as a weapon against locust plagues.

His first observations were made in the Yucatán peninsula in Mexico, but it was in Argentina, between 1911 and 1914, that he obtained his main results. The initial discovery was that of a *coccobacillus* present in the intestines of crickets and locusts, provoking the rapid death of the animals, first called *C. sauterelle* and later *C. acridiorum*. He increased its virulence by transferring it from animal to animal of the species that he wanted to eradicate, cultivated it to obtain large amounts of material, and dispersed the culture on vegetation in close proximity to the groups of crickets and flights of locusts. The dissemination of the disease was favoured by the habit crickets have of eating their dead fellows. He carefully described his observations and protocols in a series of publications (d'Hérelle 1911, 1914). The same bacterial weapon was used in Algeria (Sergent and Lhéritier 1914), Colombia, Cyprus, and Tunisia, and similar positive results were obtained. Not only were the insects destroyed, but the effects were still visible one year later.

The scientific context was favourable. Many attempts had been made in previous years and decades to develop biological control of invading species, as well as of pests, in the United States and in Europe, although d'Hérelle was one of the rare biologists to use microorganisms.

Two conclusions emerged from this work that would prove important for d'Hérelle's future work on the bacteriophage and its use in human therapeutics. The first is that antagonism between organisms is a general phenomenon in nature. Biological control is not a creation of biologists, but the use of mechanisms already operating in nature. This explains why the discovery of the bacteriophage was not anticipated by d'Hérelle, but easily found its place in his vision of the biological world. It explains why d'Hérelle had never any doubt about the living nature of the bacteriophage. His use of *coccobacilli* against locusts had also shown him that the same microbe was able to adapt and to develop on different species of

insects. He transferred this observation to the bacteriophage, which he always considered as a unique species.

When, in 1917, while studying a dysentery epidemic in troops, d'Hérelle discovered in the faeces of soldiers recovering from the disease a filtrating invisible agent able to reproduce in *bacilli* of dysentery and to kill them, he was in familiar scientific territory (d'Hérelle 1917).

5. The deep historical context of d'Hérelle's work

D'Hérelle's ideas on the bacteriophage cannot be fully understood without a description of the deeper context, of his attachment to the Pasteurian tradition and of the links that he kept with the Pasteur Institute, despite his adventurous life and conflicts. He had a deep admiration for the work of Pasteur himself and of Ilya Mechnikov, and an ambition to reach comparable heights. I will emphasize two facets of this legacy of Pasteur and of the Pasteurian tradition. The first has already been briefly mentioned by William Summers (Summers 1999); the second has been extensively studied by Richard Burian and Jean Gayon (Burian and Gayon 1991), and more recently by Laurent Loison through his study of the influence of neo-Lamarckism on French biology (Loison *et al.* 2017).

Jean Chaussivert and his colleagues have described a little known episode of Louis Pasteur's life: how he rapidly responded to the internationally advertised reward created in 1887 by the Government of New South Wales in Australia for a biological method to eliminate rabbits from the colony (Rountree 1988; Chaussivert 1991). Pasteur had already considered the use of biological methods in the case of phylloxera. Mechnikov had also acquired experience of biological control in Russia, and his efforts to delay ageing in humans were based on the competition between good and bad microbes in the gut.

Pasteur initiated experiments near Reims to demonstrate the effectiveness of the agent of chicken cholera in eliminating rabbits. He made contact with the authorities of New South Wales and, in February 1888, sent his nephew Adrien Loir to Sydney.

The difficulties encountered have been carefully described (Chaussivert 1988): the fear of the risks associated with the use of microbes on the part of Australian authorities, as well as the issues originating from the immensity of the territories that had to be treated. This did not prevent the work of what was the first overseas Pasteur Institute (1888-1898) from being fruitful – in the characterization of cattle diseases, and in the production of vaccines against anthrax and pleuropneumonia. These were later replaced by vaccines produced in Australia (Todd 1988). A new Pasteurian mission to eradicate rabbits with chicken cholera was organized in 1906 by Jean Danysz, with no more success (Rountree 1988).

Amusingly, the parallel between the work of Pasteur and the work of d'Hérelle was not explicitly made by d'Hérelle himself, but by an Argentine writer, Arturo Cancela, who satirized the adventures of d'Hérelle in Argentina by converting locusts into rabbits in his novel published in Madrid in 1923. The activities of the so-called Professor Herrlin were considered as successful – no rabbits were visible following his intervention - before it was concluded there had never been any rabbit in Argentina! Strangely, according to Summers, d'Hérelle found this little story 'very humorous' (Summers 1999, p. 42). Maybe, for d'Hérelle, the parallel implicitly made between him and Pasteur was more important than the ambiguous description of the results!

The second dimension of the deep historical context was the success of neo-Lamarckism among French biologists between the 1880s and the middle of the 20th century, and the close relation it had with the physiology of Claude Bernard on the one hand and the Pasteurian method of attenuation on the other. Although d'Hérelle only episodically discussed mechanisms of heredity, he shared with neo-Lamarckians many convictions: the description of a living organism as a system able to adapt and to assimilate, a legacy of Claude Bernard; the importance attributed to the colloidal state of matter – a way to revisit with modern chemistry the importance attributed by Bernard to protoplasm; a mistrust in cell theory, also inherited from Bernard, that led him to state later that the bacteriophage was an organism, but not a cell.

6. Conclusions

I have shown how different were the contexts of the discovery of the bacteriophage by Twort and d'Hérelle. The discovery was for d'Hérelle a prolongation of his previous work on the biological control of locust plagues, which helped him to interpret immediately the behaviour of the bacteriophage as that of an independent organism. The neo-Lamarckian and Pasteurian traditions also represented a favourable niche for his discovery and interpretation of the bacteriophage, although the ideas of Twort on life and the importance of protoplasm were not very different.

More than the legacy of a theoretical framework, this favourable context was the product of earlier research experiences, and the consequence of d'Hérelle's personality. More important for d'Hérelle than the inheritance of acquired characters was his daily practice of attenuation and exaltation of *coccobacilli* and bacteriophages. His profound conviction that the bacteriophage was an organism originated in the similar behaviours of bacteria and bacteriophages. This identity of practice was what permitted d'Hérelle to identify the bacteriophage and to give it its attributes.

Similarly, the strong ambition of d'Hérelle, his self-confidence (Summers 2013) and his hope to obtain results as important as those obtained by Pasteur, explain the

(important) role that he attributed to the bacteriophage better than any theory. The bacteriophage had to be the perfect universal weapon against infectious diseases. Its effects were not anecdotal, but had their foundations in the general economy of the living world, in the permanent fight between organisms. This explains the ambiguous link that d'Hérelle made between the bacteriophage and immunity, suggesting that the former had a major role in the latter, a more significant role than the humoral and cellular forms of immunity painfully studied by his Pasteurian colleagues.

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