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"Who Discovered Bacteriophage?"

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INTRODUCTION

Although most authors state that Twort in 1915 and d'Herelle in 1917 independently discovered bacterial viruses (8, 9, 26), some have made allusions which suggest that d'Herelle may not have been altogether honest in claiming to have had no knowledge of Twort's 1915 discovery when he published his 1917 work. In his excellent book, *The Molecular Biology of Bacterial Viruses*, Gunther Stent writes: "Gratia [sic] drew attention to Twort's forgotten—or, rather, never noted"—discovery (33). And in his introduction to *Phage and the Origins of Molecular Biology*, a similar ambiguity appears: "Bacterial viruses were discovered in 1915 by the English microbiologist F. W. Twort, and two years later—perhaps independently, perhaps not—by the French-Canadian F. d'Herelle" (34). Stent had no proof that d'Herelle had been dishonest (G. Stent, personal communication), but was very much influenced by André Lwoff who *felt* that d'Herelle may have been dishonest (G. Stent, personal communication; A. Lwoff, personal communication). Since I thought the idea that d'Herelle had had knowledge of Twort's work was completely incompatible with the joyous enthusiasm with which he described his discovery (18), I investigated the matter to see how this accusation might have arisen and to see whether, indeed, I could discover who discovered bacteriophage. It would seem, on the surface, that who discovered what would be a relatively simple matter to resolve. Actually, though, the pages of the history of science are strewn with disputes, often sordid, over priority (31). It appears that the territorial imperative is as real in the Kingdom of Science as it is in the jungles of Africa.

To unravel the many possible reasons for these "territorial" disputes is rather more difficult than to prove that they exist. Their origins undoubtedly lie in a complex of unresolved questions involving the sociology and philosophy of science. The disputes may, for instance, arise from the actual frequency of simultaneous discovery. But to understand why so many discoveries are made by different individuals independently at the same time depends on an intricate philosophical analysis of the way in which science progresses. Discoveries may not be simple events occurring at definite time points, but rather may result from more elaborate extended processes involving large numbers of people. This has been extensively discussed by Thomas Kuhn (28). In this view, claims to priority would be somewhat artificial, if not meaningless.

One could also invoke egotism as the cause of the many acrimonious controversies over priority that scientists have engaged in. The sociologist, Robert Merton, has rejected this idea, however, and sees the disputes as "signposts announcing the violation of the social norms" of the scientific establishment. In his view, originality in science is at a premium, and rewards come from demonstrations of originality. But humility and allocation of credit is also an institutional norm. Under the stresses imposed by the system, the balance between these opposing values may be upset, and pathogenic concern with original discovery may result in "contentiousness, self-assertive claims, secretiveness . . ." (31).

Many disputes also surely arise from the lack of a clear-cut definition of what constitutes discovery. Is, for instance, an observation a discovery, or must all the ramifications of the observations be recognized to constitute a discovery? These questions, however—what is discovery and what is the origin of the fervor to be first—are beyond the scope of this paper. As it turns out, even the question posed in the title is not answered. Instead, it is my intent to present some of the heretofore neglected circumstances surrounding the discovery of bacteriophage and, I hope, to clear the name of Felix d'Herelle from the aspersions that have been cast upon it.

(This paper is part of an address entitled "The Filterable History of Discovery," presented at the University of Florida on 2 March 1976 as one of the President's Scholars Lecture Series.)

TWORT'S DISCOVERY

F. W. Twort, the son of a country doctor and a hard-working mother, was trained in medicine at St. Thomas Hospital in London, but as the fortunes of his family were small he was unable to pursue his keen interest in pathology. "It was imperative," relates Twort in his article, "The Discovery of the Bacteriophage" (37), "that I should earn at least sufficient to be able to pay for lodgings and food. Accordingly, I accepted the first paid post available, which was that of Assistant Superintendent of the clinical laboratory at the hospital." The post enabled him, nonetheless, to gain experience in pathology, and in 1909 Twort was appointed Superintendent of the Brown Institution, London University. The Brown Institution had been founded in 1871 to provide a hospital "for the care and treatment of Quadrupeds or Birds useful to man." In his capacity as director, Twort was allowed to carry out research in any branch of pathology or bacteriology, "provided expenditure could be kept within the limits of the small income available." He chose to study the growth requirements of primitive forms of life. He had one success, obtaining an essential substance that would allow Johne's bacillus, the causative agent of a serious disease of cattle, to grow in artificial medium. His essential substance has since been identified as vitamin K (20, 37).

He then went on to perform experiments intended to indicate how viruses could be grown in artificial media. This seems an unfortunate avenue of research to us as we now know that viruses can only grow, if indeed we can say they grow at all, inside living cells. But Twort was proceeding upon a not unreasonable hypothesis which was that, since viruses were the smallest, hence simplest, forms of life, they must have, at one time in evolutionary history, been able to grow in a medium devoid of any living matter.

As Twort explains in his famous note to the *Lancet* on 4 December 1915 (35), "attempts to cultivate these (viruses) from such materials as soil, dung, grass, hay, and water from ponds were made on specially prepared media. It is impossible to describe all these in detail but generally agar, egg, or serum was used as a basis, and to these varying quantities of certain chemicals or extracts of fungi, seeds, and etc., were added." He would then inoculate these various media with extracts of his various soils, dung, or sera, which had been filtered through candle filters to remove all bacteria. Every one of his hundreds of experiments was negative. He never succeeded in growing a filter-passing agent on artificial media. He did, however, observe something else. He had inoculated an agar medium with some fluid (unfiltered) commonly used for smallpox vaccinations. He noticed that although the vaccinia virus did not grow, a bacteria—a micrococcus—did grow. The bacteria, however, appeared to be afflicted with some disease—"inoculated agar tubes often showed watery-looking areas, and in cultures that grew micrococci it was found that some of these colonies could not be subcultured, but if kept they became glassy and transparent." In retrospect, this phenomenon, which came to be known as the glassy transformation of Twort, seems exceedingly strange. It is so rare that, in the more than 14 years that I have worked with bacteria and their viruses, I have never observed it—at least that I know of. Furthermore, I have seen only one reference in the literature to a similar phenomenon, and that was Sir Alexander Fleming's observation that what was later found to be penicillin caused colonies of staphylococcus to appear like "drops of dew" (2). Twort, however, made some very interesting observations about the glassy transformation. (i) The affected colonies would not grow on any medium. (ii) Examination of the glassy areas revealed only minute granules and no bacteria. (iii) If a pure culture of micrococcus was touched with a small portion of one of the glassy colonies, the growth at the point touched started to become transparent and gradually made the whole colony transparent. (This is the startling part of his observation as regards bacterial viruses. Usually by the time bacteria have grown long enough to form a visible colony, they have reached a phase such that they cannot be attacked by a virus.) (iv) After filtration of the glassy material through a Chamberland candle, it retained its ability to cause the "glassy transformation." (v) The "transformation" could be conveyed to fresh cultures for an indefinite number of generations.

Twort concluded that the cause of the glassy transformation was an infectious, filterable agent that killed bacteria and in the process multiplied itself. He could have very logically concluded that he was dealing with a bacterial virus. But he does not do so. He says:

> from these results it is difficult to draw definite conclusions. . . . [This] may be living protoplasm that forms no definite individuals or an enzyme with the power of growth. . . . In any case, whatever explanation is accepted, the possibility of its being an ultra-microscopic virus has not been *definitely* disaproved because we do not know for certain the nature of such a virus.

Then a little later, "On the whole it seems probable, though by no means certain, that the active transparent material is produced by the micrococcus, and since it leads to its own destruction and can be transmitted to fresh healthy cultures it might almost be considered as an acute infectious disease of micrococci." At the end of the paper he laments, "I regret that financial considerations have prevented my carrying these researches to a definite conclusion" (35).

Here, as happened also with the discovery of plant viruses and animal viruses, we have a remarkable discovery faced by its discoverer with uncertainty. Although it was by this time known that there were filterable viruses that could grow in either plant cells or animal cells, Twort was reluctant to believe he had discovered a bacterial virus – if indeed he had – although he did consider this a possibility. Hence he considered Beijerinck's old idea of a fluid form of life, introduced to explain the filterability of the agent of tobacco mosaic disease (3), and also the possibility that the micrococcus was producing an enzyme that could grow and multiply. This latter idea, which seems ridiculous now, was the focal point of a controversy that raged in the scientific literature for many years. The controversy did not start immediately, however. In fact, Twort's paper went unrecognized until 26 March 1921 – 5 years, 3 months, and 22 days.

D'HERELLE'S DISCOVERY

Felix d'Herelle, the man who was heralded for 4 years as the sole discoverer of bacterial viruses – or bacteriophage, as he called them – lived a life that was more befitting of a Magellan, a true explorer, than a mere bacteriologist. Born in 1873 in Montreal of a French-Canadian father and a Dutch mother, he was educated in France and Holland, and during his life traveled and worked in Guatemala, Mexico, South America, Egypt, Algeria, Tunisia, Indochina, and Russia. He was a professor at Yale for 5 years, and an associate of his there has recently said of him that everywhere d'Herelle was, there were fireworks (Florence Mack, personal communication). The fireworks materialized rather unfortunately in Russia, where d'Herelle went several times during the 1930s to found institutes for the study of bacteriophage. During one of his trips his trusted associate, Eliava, was arrested and shot (1, 7, 29, 32). He left Yale for reasons that are not entirely clear, and "several confidential letters exchanged between Dean Winternitz and Professor d'Herelle cannot be released" (A. Ebbert, personal communication).

It was in Mexico that d'Herelle first observed what later came to be called bacteriophage. His account of this discovery written in 1949 – about 40 years after he had made the discovery – is rarely equalled in the scientific literature of today for its ingenuous enthusiasm (18). He said:

> In 1910, I was in Mexico, in the state of Yucatan, when an invasion of locusts occurred; the Indians reported to me that in a certain place the ground was strewn with the corpses of these insects. I went there and collected sick locusts, easily picked out since their principal symptom was an abundant blackish diarrhoea. This malady had not as yet been described, so I studied it. It was caused by bacteria, the locust coccobacilli, which were present almost in the pure state in the diarrhoeal liquid. I could start epidemics in columns of healthy insects by dusting cultures of the coccobacillus on plants in front of the advancing columns: the insects infected themselves as they devoured the soiled plants.
>
> During the years which followed, I went from the Argentine to North Africa to spread this illness. In the course of these researches, at various times I noticed an anomaly shown by some cultures of the coccobacillus which intrigued me greatly, although in fact the observation was ordinary enough, so banal indeed that many bacteriologists had certainly made it before on a variety of cultures.
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> The anomaly consisted of clear spots, quite circular, two or three millimetres in diameter, speckling the cultures grown on agar. I scratched the surface of the agar in these transparent patches, and made slides for the microscope; there was nothing to be seen. I concluded from this and other experiments that the something which caused the formation of the clear spots must be so small as to be filtrable, that is to say, able to pass a porcelain filter of the Chamberland type, which will hold back all bacteria.
>
> However, the appearance of these clear patches was inconstant. I sometimes went weeks without seeing a single one, and I could not reproduce the phenomenon at will; I therefore could not study it.

In March, 1915, during the first World War, a large invasion of locusts appeared in Tunisia, threatening to destroy the harvests which were then so vital; I was given the job of starting an epidemic amongst them. As the result of the infection there was a considerable mortality, and, even more interesting, when the following year all the rest of North Africa was again invaded, Tunisia remained free.

In the course of this campaign, I again observed my clear spots, and before returning to France I stayed for a time at the Institut Pasteur in Tunis, to investigate their significance.

I showed them to Charles Nicolle, then director of the Institute, and he said to me: 'That may be the sign of a filtrable virus carried by your coccobacilli, a filtrable virus which is the true pathogenic agent, while the coccobacillus is only a contaminant.' So I filtered emulsions of cultures grown on agar and showing the clear spots, and tried to infect healthy locusts with the filtrate, but without result.

On my return to Paris in August 1915, I was asked by Dr. Roux to investigate an epidemic of dysentery which was raging in a cavalary squadron, then resting at Maisons-Laffitte. I thought the hypothesis put forward for the locusts' illness might be helpful in understanding human dysentery. I therefore filtered emulsions of the faeces of the sick men, let the filtrates act on cultures of dysentery bacilli and spread them after incubation on nutritive agar in petri dishes: on various occasions I again found my clear spots, but the feeding of these cultures to guinea pigs and rabbits produced no disease.

At this time we often got cases of bacillary dysentery in the hospital of the Institut Pasteur in Paris. I resolved to follow one of these patients through from the time of admission to the end of convalescence, to see at what time the principle causing the appearance of the clear patches first appeared. This is what I did with the first case which was available.

The first day I isolated from the bloody stools a Shiga dysentery bacillus, but the spreading on agar of a broth culture, to which had been added a filtrate from the faeces of the same sick man, gave a normal growth.

The same experiment, repeated on the second and third days, was equally negative. The fourth day, as on the preceding days, I made an emulsion with a few drops of the still bloody stools, and filtered it through a Chamberland candle; to a broth culture of the dysentery bacillus isolated the first day, I added a drop of the filtrate; then I spread a drop of this mixture on agar. I placed the tube of broth culture and the agar plate in an incubator at 37°. It was the end of the afternoon, in what was then the mortuary, where I had my laboratory.

The next morning, on opening the incubator, I experienced one of those rare moments of intense emotion which reward the research worker for all his pains: at the first glance I saw that the broth culture, which the night before had been very turbid, was perfectly clear: all the bacteria had vanished, they had dissolved away like sugar in water. As for the agar spread, it was devoid of all growth and what caused my emotion was that in a flash I had understood: what caused my clear spots was, in fact, an invisible microbe, a filterable virus, but a virus parasitic on bacteria.

Another thought came to me also: 'If this is true, the same thing has probably occurred during the night in the sick man, who yesterday was in a serious condition. In his intestine, as in my test-tube, the dysentery bacilli will have dissolved away under the action of their parasite. He should now be cured.

'I dashed to the hospital. In fact, during the night, his general condition had greatly improved and convalescence was beginning.' (From F. d'Herelle, The bacteriophage, Sci. News 14:44–59, 1949. Copyright © Penguin Books Ltd 1949. Reprinted by permission of Penguin Books Ltd.)

In d'Herelle's first communication on bacteriophage, published in 1917 and entitled "On an Invisible Microbe Antagonistic to Dysentery Bacteria" (13), he does not mention his observation of the clear spots in the cultures of the locust bacteria, and I think he later came to regret this fact. He tells only of his work in Paris with dysentery. The main observations reported were that an agent capable of killing the dysentery bacterium appeared in the intestines of persons recovering from dysentery, but not from individuals suffering from the acute stage of the disease or from normal individuals; that this agent could be filtered through a Chamberland filter and still be active; that it could be serially transmitted and hence must be a "living germ"; that it would not grow on any artificial medium, and therefore had to be an obligatory parasite, a "bacteriophage"; and (*mirabile dictu*) that this antagonist could "immunize" rabbits against a lethal dose of the dysentery bacteria. Although, for a historical record, d'Herelle's conclusion that he had found a living organism that would grow only in bacteria (a bacterial virus) is the most noteworthy, for d'Herelle and many others it was this latter observation, that this "antagonist" might be the agent of immunity to bacterial disease, that was the most thrilling. He ends his paper by expressing his belief that this "immunity" may be a general phenomenon. He does not mention the work of Twort that had preceded his by about a year and a half; in fact, there are no references at all in the paper, although this was not uncommon for papers presented to the Academy of Science at that time. Beijerinck, who is often credited exclusively with the original discovery of viruses, had also not cited the earlier work of Ivanowski (3) – work that had preceded his by 6 years.

THE DISCOVERY OF TWORT'S DISCOVERY

For the next 4 years, d'Herelle lived a life that every scientist must dream of. During those years, people in all parts of the world were isolating and studying agents of transmissible bacterial lysis – bacteriophage – and most people referred to what they were studying as the "phenomenon of d'Herelle." It was hoped by many that these bacterial killers could be used therapeutically in the treatment of bacterial disease, and it seemed as if Pasteur's idea of microbes fighting microbes or Erlich's "magic bullet" had at last materialized. Hundreds of people cited d'Herelle's work, and although he may not have been universally regarded, he was certainly universally acknowledged. Then, in 1921, a paper appeared that was to end his glory. The paper, by J. Bordet and M. Ciuca and entitled "Remarks on the History of Research Concerning Transmissible Bacterial Lysis," was presented at the 26 March 1921 meeting of the Belgian Society of Biology (5). Trouble had actually started in 1920. A dispute had arisen over the true nature of the "d'Herelle phenomenon." Kabeshima (27), working on immunizing animals using phage, had come to the conclusion that the lytic agent could not be a living being but was a chemical catalyst produced from a "pro-ferment" found in all microbes. The pro-ferment could, supposedly, be activated by an outside influence or by the catalyst itself. Bordet and Ciuca (4) had then shown that a leukocytic exudate could cause normal bacteria to produce an agent of transmissible lysis. They hypothesized that the bacteria were first stimulated by leukocytes to produce a lytic enzyme and that this enzyme could then, in the absence of the leukocytes, stimulate other bacteria to produce more of the enzyme. This idea was similar to Kabeshima's but also to Twort's idea that the transmissible lysis was caused by an enzyme with the power of growth. Hence, in their March 1921 paper (5), Bordet and Ciuca say:

> it is currently admitted that d'Herelle has been the first to observe the lysis which he attributes to a bacteriophage, but which we believe to have shown represents an autolytic phenomenon that one can start in perfectly normal microbes by a leucocytic exudate. The burden of an exact history makes it necessary for us to cite a previous work which d'Herelle *has not known* [italics mine] and that we ourselves have been ignorant of until now that contains the observations that d'Herelle has made. This remarkable work by F. W. Twort appeared in Lancet in 1915, that is to say, two years before the research of d'Herelle.

They go on to describe Twort's observations and his various interpretations. Concerning the possibility raised by Twort that what he observed was due to an autolytic enzyme produced by the microbe itself, they say: "this author has expressed the idea for which we have proved the solid basis." They conclude: "without wanting to diminish the interest of the observations of d'Herelle we believe that it is a duty to recognize the incontestable priority of Twort in the study of this question" (5). It was obviously a duty they took delight in performing!

A very similar thing had happened to Beijerinck when he published his paper on filterability of tobacco mosaic virus in 1898 (3). Ivanowski pointed out, in a short communication in 1899, that he had observed the same thing 6 years earlier, but in this case Beijerinck had apologized. d'Herelle, however, did not react as Beijerinck had.

On 11 May 1921, d'Herelle read his "defense" to the meeting of the Society of Biology in Paris (14). The gist of the paper was that Twort was dealing with something else – not bacteriophage. He says "I have been able to find only two references in the scientific literature that could pertain to the question of bacteriophage. The first is that of Hankin (Ann. de l' Institut Pasteur, 1896) who states that the water of certain rivers of India possesses a bactericidal action . . . no doubt bacteriophage has been the cause." The second reference that d'Herelle admits may pertain to bacteriophage is Twort's paper of 1915. He points out that Bordet and Ciuca have recently cited Twort's work but have possibly misinterpreted a part of it. Although Bordet and Ciuca claim that Twort showed that his lytic activity was transmissible in series in a suspension of bacteria, d'Herelle claims that he (Twort) made "not the slightest allusion to such a phenomenon" (14). What Twort does say is the following: "This condition or disease of the micrococcus when transmitted to pure cultures of the micrococcus can be conveyed to fresh cultures for an indefinite number of generations" (35). All Twort's other observations had been made on cultures of micrococcus growing on agar, so there is no reason to think he did this experiment in liquid cultures, as Bordet and Ciuca say he did. So it is probably true that they did misinterpret Twort, but the misinterpretation had very little to do with the substance of their paper – namely, that Twort had observed transmissible lysis of bacteria. This argument over a minor point, which takes up a good part of d'Herelle's paper, was only the first of many spurious arguments that were to be used by both sides in an escalating war between the Paris phage-ologists and the Belgian phage-ologists. (The ridiculous arguments seemed to reach their epitome when, at a July 1922 meeting in Glasgow, Gratia, arguing that phage is not living says, "fire is not living yet fire is endowed with power of reproduction," and later: "when we pour out a glass of soda water there appear on the wall of the glass small round bubbles of gas, the size of which increases exactly as the so-called colonies of bacteriophage, and yet gas is not a virus" [23].) The remainder of d'Herelle's 1921 paper is largely a summary of Twort's work as reported in 1915, after which he says, somewhat dramatically, "the bacteriophage – is it the cause of the very interesting phenomenon described by Twort?" He answers himself, "it is possible but 'peu vraisemblable' " (14). His reasons for thinking so seem to be largely intuitive, although he does cite a difference between the thermal inactivation points of the agent that Twort isolated and his own bacteriophage preparations. He ends his paper by pointing out that "the intensity of bacteriophage action is so 'brutal' that many bacteriologists ought to have seen it in the course of their researches but have neglected it because it was for them incomprehensible" (14).

Who were these many bacteriologists who had seen the phenomenon but neglected it? Was one of them perhaps a young bacteriologist working in Mexico to save the world from plagues of locusts?

d'Herelle's defense of himself seems to have had the effect of a declaration of war on the Belgian microbiologists. A formidable ally, André Gratia, joined Bordet's forces, and in November 1921, a note by Gratia and Jaumin was read to the Belgian Society of Biology (25). The title was "Identity of the Phenomenon of Twort and the Phenomenon of d'Herelle."

Gratia and Jaumin had isolated from vaccinia pulp (the same material Twort had started with) an agent that had a "marked inhibiting and dissolving action on staphylococci" and showed that the agent could be transmitted indefinitely from one culture to another. Their note begins by saying that they have confirmed the observations of Twort, and furthermore, obtained a lytic principle that has permitted them "to reproduce with Staphylococci all the particulars of the phenomenon for which d'Herelle claims priority and which he attributes to bacteriophage." For evidence, they refer to earlier papers which, they say, leave no doubt as to the identity of Twort's phenomenon and d'Herelle's phenomenon – "an identity which the latter author thought he could contest." An examination of their earlier papers (21, 22) reveals that it is far from certain that they had proved the two phenomena were the same. They had, indeed, isolated a lytic principle – but they had isolated it from some "small, clarified areas" of their agar slants, not from colonies that started to grow and then became glassy. Little attempt was made to repeat the identical experiments of Twort. They mention the word "glassy" once, but more often talk about irregular colonies as being the ones that contain the lytic principle. One gets the feeling that the word "glassy" was thrown in somewhat surreptitiously.

Their November report (25), although entitled so as to lead one to believe they had conclusively reconciled Twort's observations with d'Herelle's, is, in actual fact, an attempt to identify a lytic principle, a phage, they had isolated from vaccinia pulp (not necessarily what Twort had isolated) with a staphylococcal bacteriophage they had isolated previously and that they believed to be a multiplying enzyme. It becomes apparent that had Twort claimed to have discovered a "living germ – an obligatory bacteriophage" (16, 17) as d'Herelle had, and not an enzyme that could reproduce itself, his work would have remained undiscovered for even longer than it did. There seems to be little doubt that it was because Twort favored the hypothesis of the Belgian group that he was discovered. (This view has also been expressed by P. Fildes [20].)

THE CONTROVERSY GROWS

In the age of molecular biology, when it is known rather precisely what (although not why) a virus is, the hypothesis of Bordet's group seems, to say the least, unsophisticated. d'Herelle's criticism does not seem unreasonable:

> The leucocytic exudate induces the 'nutritive vitiation' which results in the lysis of the bacteria in the first tube of the series, but in the following ones the same effect will be produced, no longer by the leucocytic exudate, which will of necessity have disappeared in the first tubes because of the dilution, but by the bacterial lytic ferment alone. Bordet and Ciuca seem to find this substitution of cause entirely logical, when in reality it is contrary to all that we know (17).

However, it is probable that the merits of the idea (or lack thereof) had very little to do with the heat generated by the controversy between d'Herelle and Bordet. Whether or not all scientists can be said to be largely motivated by the possibility of widespread recognition, it is certainly true that, in the controversy over whether agents of bacterial lysis were "living germs" or "multiplying enzymes," there were no tiny egos involved. d'Herelle, who is now acknowledged to have been, for the most part, correct (19), at least as regards virulent phage, may have been particularly abrasive.

In writing of d'Herelle shortly after his death, one of his associates, Pierre Lepine, said: "Felix d'Herelle was a curious and magnetic personality. Lively, sometimes irascible, he was a faithful and sure friend. If he sometimes gave to those who knew him but little the impression of a difficult character, those who knew him well have not ceased to feel his affability and joyous ardor in his work" (29).

That he was easily aroused in his own defense there is no doubt. A mere glance at the Table of Contents of his first book (16) reveals that he had no patience with those who disagreed with him. Among the subtitles of his chapter, "The Nature of the Bacteriophage" (a chapter added in 1922 for the English edition), are the following: experimental proofs of the living nature of the bacteriophage; refutation of the hypothesis of Kabeshima; refutation of the hypothesis of Bordet and Ciuca; refutation of the hypothesis of Bail; refutation of the hypothesis of Salimbeni; conclusions. In his conclusions he states, "all of the authors who have advanced hypotheses other than that of an ultramicrobe parasitizing the bacteria have forgotten that a hypothesis ought always to account for the entire mass of facts."

In the summer of 1922, d'Herelle, Twort, Bordet, and Gratia were invited to speak at a British Medical Association symposium on the bacteriophage held in Glasgow. d'Herelle spoke first—and longest. He says again that many authors have neglected the whole assembly of facts—a remark sure to win him few friends. He presents four hypotheses on the nature of bacteriophage and proceeds systematically to eliminate all but one: the one to which "I have held since my first publication" (15).

Twort's presentation was very much a reiteration of his 1915 paper in which he tries very hard to walk the thin line between Bordet and Gratia on the one hand and d'Herelle on the other. He views his original results as "evidence not only against [d'Herelle's] view that the lytic agent is a living organism, but also against the view of Bordet and Ciuca" (36). For d'Herelle, anyone that was not with him was against him. Hence, in his second book published in 1926 (17), the hapless Twort comes under attack. In his first book d'Herelle had mentioned Twort's paper as bearing on the subject of bacteriophage (16). In 1921 he had also allowed that Twort's observation might bear on the subject of bacteriophage (14). But in his 1926 book he states that "as is evident, the phenomenon observed by Twort and the phenomenon of bacteriophagy present two entirely different aspects ... those authors who have likened the phenomenon of Twort to bacteriophagy have restricted themselves to affirmations only, without offering any supporting proof whatever." He states that Twort refused to discuss the issue with him publicly, both in 1922 and in 1923 and that "it is difficult to avoid the conclusion that if Twort refrains from such a discussion it is simply because the facts revealing the dissimilarity of the two phenomenon are indisputable."

D'HERELLE IS DISCREDITED

d'Herelle's 1926 book was notable in another respect. It was in this book that d'Herelle first published in detail the story of his finding bacteriophage in Mexico. He had actually mentioned seeing plaques with his locust bacteria in a chapter added to the English edition of his first book in 1922 (16), although he had mentioned it in his historical introduction to the French edition written in 1921. It seems that the rediscovery of Twort had a great deal to do with the rekindling of d'Herelle's memory. During the time between the publication of the French edition and the English edition, Bordet's and Gratia's attacks had begun in earnest and, by 1925, when d'Herelle wrote his second book, the attacks had escalated. With the attacks came increasing insistence that Twort and not d'Herelle had first discovered bacteriophage. Now d'Herelle's recounting of his first seeing the plaques in 1910 became considerably more convincing.

It is my hypothesis that with d'Herelle's increasing insistence that he was the sole discoverer of bacteriophage, and the sudden appearance of the story of his 1910 work, came the accusation that he had known of Twort's work. It can certainly be said that, by introducing the story of his 1910 observations, d'Herelle acted as a guilty man. If he had truly not known of Twort's work, would he not have been apologetic upon discovering that Twort had earlier made a similar observation? Some will say that only a guilty man would go to such lengths to prove his innocence. One need not, however, invoke guilt to explain d'Herelle's claim to have discovered phage before Twort. d'Herelle was at this time an extremely beleaguered man. Whether or not Twort had seen the effects of phage first, it was d'Herelle who, by the sheer volume of his work, had brought the phenomenon to the attention of the world and had made research with bacteriophage one of the most exciting fields to work in during the 1920s. He developed the quantitative plaque assay and quantitative dilution technique and, by use of these, described the process of bacteriophage growth, which is remarkably "close to the picture we have today" (19). In spite of this, he was cruelly and viciously attacked. His triumph had evaporated – the d'Herelle phenomenon had become the Twort-d'Herelle phenomenon – not even the d'Herelle-Twort phenomenon. Attempts were made to make him look somewhat a fool (23). For a man who was extremely proud of his associations with the tradition of Pasteur and of his discussions about phage with Albert Einstein (17), this must have been bitter medicine indeed. Furthermore, d'Herelle was a very sincere man who, I have no doubt, honestly believed that Twort had observed something else. In this case, it would be immaterial whether or not he had seen Twort's paper.

DID D'HERELLE DISCOVER PHAGE IN 1910?

The role of communication in the establishment of priorities not withstanding, the question does remain – did Twort or did d'Herelle observe bacterial viruses first? Did d'Herelle see "taches vierges" – clear spots – on the cultures of the locust coccobacillus? He did, indeed, report on a disease of locusts that he observed in the Yucatan peninsula of Mexico. This was in a note presented to the Academy of Science in 1911 (10). But he does not mention the clear spots. Nor does he mention them in his very scholarly papers on the locust coccobacillus published in 1914 (11, 12). That he does not mention them, does not, of course, mean he did not see them. Remember the many bacteriologists who had seen the phenomenon but, not knowing what it was, had neglected it (14). Unfortunately, the one person who could tell us the true story lies buried near his home on the outskirts of Paris. Because d'Herelle was of Canadian birth he was incarcerated by the Germans during their occupation of France. He was freed at the end of the war, but was a sick man. He died on February 22, 1949. Twort and Gratia both died the next year.

CONCLUSIONS

We can see that, as Thomas Kuhn predicted, "research into the history of science makes it harder, not easier, to answer questions like when was oxygen discovered? Who first conceived of energy conservation?" (28). Who discovered bacteriophage? The question is unanswerable. On the other hand, we can see that there is no evidence that d'Herelle "stole" Twort's discovery. As Twort's paper seems to have been generally unnoticed until 1921, there is no reason to think that d'Herelle had seen it in 1917. Even Bordet and Ciuca said in 1921 that d'Herelle had "not known" of Twort's work (5). So it seems quite logical to conclude that, indeed, he had not; and that, if Twort did discover bacteriophage in 1915, then d'Herelle did so independently in 1917, if not in 1910. We can rightly question whether or not Twort actually did "discover" bacteriophage at all, since he certainly did not conclude that what he had observed was a bacterial virus. It was Bordet, in favoring Twort's multiplying enzyme theory, who made the priority claim for him. However, since Twort did consider the idea of a bacterial virus, and since temperate phage may appear to have some of the properties of multiplying enzymes, it would seem ungracious and unfit to give him no credit.

It is tempting to do a "Mertonian" analysis as to why the dispute regarding the true discoverer of phage was so heated. It could be said that in refusing to credit Twort, even when his work was brought to light, that d'Herelle violated one of the institutional mores of science – that of humility. Hence he aroused the moral indignation of the scientific community and had to be censured. But even in their first publication, "Remarks on the History of Research Concerning Transmissible Bacterial Lysis," the tone of Bordet and Ciuca was anything but conciliatory. They, in a sense, threw down the gauntlet. It seems more likely that the fight was over territory – who will be the king?

Yet even if we cannot decide who was the true discoverer of bacteriophage, or add significantly to the sociology of priority disputes, we can draw some conclusions regarding the effect of these disputes on the rapid advancement of science. The field of bacteriophage research remained in a dire state of disunity well into the 1930s. The discovery of colicins and lysogenic bacteria served only to heighten the already existing confusion. (See André Lwoff's excellent review in this same journal in 1953 for a discussion of this era [30].) In spite of an abortive attempt by Burnet and McKie in 1929 (6) to reconcile the two aspects of baceriophage phenomena, the temperate aspect and the virulent aspect, Gratia in 1932 had to, in what seems a rather desperate attempt, resort to a belief in spontaneous generation to try to explain all the various observations he and others had made. Said he:

> in truth, the 'pasteurien theory' [that spontaneous generation of life cannot occur], indisputable for bacteria, is perhaps not valid for 'precellular' forms of life and I recall here again the example of fire. This, in some ways resembling life, was in the eyes of our ancestors considered as being only able to come from a pre-existing flame . . . today, everyone knows that not only can fire be transmitted from one flame to another, but that it can be 'born' from the friction of a match or the 'travail' of fermentation (24).

One cannot but think that had a veritable war not been started between d'Herelle, champion of the virulent phage, and Bordet and Gratia, defenders of the temperate phage, the true nature of bacteriophage would have been realized earlier, and spontaneous generation could have been left to rest in peace.

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