1 CALLED OR RECALLED TO LIFE

DISCOVERIES AND CONCEPTIONS

> The first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism.
> —Peyton Rous, 1911

> The disappearance of the dysentery bacilli is coincident with the appearance of an invisible microbe endowed with antagonistic properties with respect to the pathogenic bacillus. This microbe, the true microbe of immunity, is an obligatory bacteriophage.
> —Félix d'Herelle, 1917

On the face of things, the circumstances under which the causative agent of sarcoma tumors in chickens and bacteriophages were discovered could not have been more different. There was a medical pathologist working on problems of cancer causation in a lab in New York City; there was a bacteriological researcher investigating bacterial dysentery in a laboratory outside Paris. For the New Yorker, at some point in the course of his investigations, to claim that he had identified a tumor-causing agent, which would later come to be known as the Rous sarcoma virus (RSV), was not in and of itself cause for undue notice, at least not at first. After all, cancer research was what he had been hired to do at the Rockefeller Institute for Medical Research.[1] That the researcher in France, as a result of his investigations on bacteria causing human dysentery, announced that he had discovered an entirely new living microbe—one, moreover, that killed the dysentery bacteria themselves—was, in contrast, quite startling and so, bound to draw attention.

Despite the differences in the specifics of their investigative prob-

lems and circumstances, the two men, working around the same time on their problems—during the second decade of the twentieth century—arrived at rather similar conclusions regarding the objects of their respective investigations. The researcher in New York characterized his find as "a minute parasitic organism," while the French investigator labeled his discovery "un microbe invisible." Basically, however, they were both saying the same thing within a few years of each other: that the quite unrelated phenomena discovered by them each were caused by viruses—namely, pathogenic agents invisible to the naked eye, or indeed, even under a regular microscope. How and why these two men arrived at such similar conclusions in their largely separate worlds is the history set forth in this first chapter.

TUMORS MOST FOWL

"Whatever you do, do not commit yourself to the cancer problem," was the advice William H. Welch gave to the young Peyton Rous, just as the latter was departing to train as a pathologist at the University of Michigan. To a fresh graduate of the Johns Hopkins University School of Medicine, such advice from one of the founding pathologists of the university appeared very sound, especially since cancer research at the time "seemed indeed the most barren of fields in which to try to make a life by finding out." But fate, in the guise of Simon Flexner, founding director of the Rockefeller Institute, evidently had other plans for this young man. For when Rous completed his training in Michigan, it was to "attempt research on cancer" that Flexner offered him a position at the Rockefeller. As Rous later recounted: "I wanted some other task. Very quietly [Flexner] asked: 'What are you working on now?' and he listened intently as I described a tiny dingle-dangle. . . . Then he gravely said: 'Do you consider this the equal of the cancer problem'—nothing cutting, nothing sardonic, just the question. Thus was the fact borne in upon me that all scientific undertakings are not free and equal, as beginners so readily assume."[2] So, Rous accepted the position and had barely begun the new job when a worried farmer visited the institute with a diseased chicken—a Plymouth Rock hen—which had "projecting sharply from the right breast, a large, irregularly globular mass."[3] Others at the Rockefeller seem to have shown little interest in the farmer's problem, but Rous seized upon it immediately as a new avenue of investigation into cancer, and perhaps even as way to vindicate his choice to go against the advice of his former mentor by breaking new ground in the field.

An initial examination of the tumor tissue from the chicken suggested that it was a sarcoma—a tumor of connective tissue—of a type hitherto not seen occurring in birds. Further studies revealed that the tumor shared many properties with sarcomas known to occur in other animals. As Rous reported: "It is formed of a single type of cell, only slightly differentiated, resembling young connective tissue cells, and possessed of an enormous proliferative energy which is exercised to the detriment of the surrounding tissues and eventually of the entire host. Growth takes place through infiltration and replacement of normal structures, as well as through expansive enlargement. Metastasis by way of the blood stream is common."[4] When bits of the tumor tissue were transplanted, either to unaffected parts of the same bird or into the breast of a healthy, tumor-free Plymouth Rock chicken—it had to be the same species—a new sarcoma developed in this location. At the end of these first preliminary experiments, Rous concluded: "So far as tested, this avian tumor closely resembles the typical mammalian neoplasms that are transplantable."[5]

Upon further investigation, however, Rous found that the new avian sarcoma differed from its other neoplasms in one very significant respect. Where the earlier tumors could be transplanted only when the material used for transplantation had intact tumor cells from the original growth, the chicken sarcoma could be induced to develop in new unaffected birds with cell-free extracts of the tumor; namely, tissue that had been processed—ground and passed through filters with pores small enough to retain bacteria—in such a way as to ensure that the filtrate was free of intact cells. Such an observation about a tumor was unprecedented; all previous efforts to transmit tumors of mice, rats, or dogs to unaffected animals using cell-free filtrates of tumor tissue had proven unsuccessful. The implication of such behavior was that whatever the identity of the tumor-inducing agent, it was extremely tiny. Also, Rous found that with each successive transplant, properties such as the rate of success of transplantation, the growth of the new tumor, and the "frequency, extent and rapidity of metastasis" increased. The results of these early experiments led Rous to the conclusion, cited in the epigraph at the outset of this chapter, that "the first tendency will be to regard the self-perpetuating agent active in this sarcoma of the fowl as a minute parasitic organism." But this conclusion was by no means definitive at this early stage, and he also acknowledged that it was "conceivable that a chemical stimulant, elaborated by the neoplastic cells, might cause the tumor in another host and bring about in con-

Fig. 1.1 Peyton Rous working at his microscope, 1923. Photograph courtesy of Rockefeller Archive Center.

sequence a further production of the same stimulant. For the moment we have not adopted either hypothesis."[6]

By the following year, Rous—soon joined in his investigations by James B. Murphy, another young pathologist from Johns Hopkins em-

Fig 1.2 Iconic image of hen with a sarcoma induced by cell-free filtrate of tumor tissue, published in the *Journal of Experimental Medicine* 12 (1910): Plate LXVI. Photograph courtesy of Rockefeller University.

barking on a research career—had found that cell-free filtrates of the chicken tumor tissue retained the ability to cause tumors in new birds despite undergoing treatments such as drying, glycerinization, and repeated freezing and thawing, which killed the tumor cells themselves.[7] In addition, and perhaps most significantly for Rous, the agent seemed to possess the ability to multiply when transmitted to new tissue: "A very little of it will give rise to a growth from which numerous others may be started, each yielding the agent in abundance."[8] Based on these studies, Rous felt more confident about his "first tendency," and therefore ended his report with a more definitive statement as to the identity of the causative agent: "Experiments with the chicken sarcoma have not yielded a method whereby a causative agent can be separated from the tumors of rats and mice. But they clearly prove that the characteristics of malignant tumors in general are compatible with the presence of a living causative agent."[9] The finding of two other chicken tumors that were also transmissible to new healthy birds via cell-free filtrates of the tumors—a bone tumor and a second sarcoma distinct from the first—further buttressed Rous's belief in the living nature of the causative agents, leading him to suggest, "The findings with the chicken

tumors largely demolish the theoretical basis on which objections to an extrinsic cause for cancer have been built up."[10] A couple of years later he followed up with an even stronger declaration, claiming, "It is perhaps not too much to say that their recognition [of the agents of these tumors] points to the existence of a new group of entities which cause in chickens neoplasms of diverse character."[11]

Although he seemed quite certain that the agents of the avian tumors were living, ultramicroscopic entities, Rous did not explicitly label the agent a "virus" in his early papers. Between 1911 and 1915, Murphy and Rous coauthored about a dozen publications on the chicken sarcoma. In all of them, Murphy would note later, "we referred to the causative factors by the non-commital [*sic*] designation of 'agents.'"[12] In the one instance Rous did use the word *virus*, the link to it as the identity of the sarcoma agents was an indirect one: "Although the filterable viruses have but recently come to attention, it is known that they are of diverse character and that . . . they can scarcely be discussed together. At present each constitutes a separate problem. This is especially true of the filterable agent which causes a sarcoma of the fowl."[13]

Certain historians have suggested that part of the reason for Rous's reticence in calling the agent a "virus" might have been because Murphy, who had collaborated with Rous on most of the avian sarcoma work until 1915, did not agree with this interpretation.[14] But this explanation is not consistent with Rous's characterization of the sarcoma agent throughout and beyond the period that he worked on the problem. The extent to which Murphy influenced the terminology in their joint papers is unclear, for as Rous observed in a letter to a friend, their disagreements never prevented him from calling "the thing a virus when lecturing."[15] He himself credited a senior colleague at the Rockefeller, T. M. Prudden, with dissuading him from calling the sarcoma agent a virus in his publications; Murphy was not mentioned as an influence.[16] Furthermore, although Murphy would distance himself from the virus theory once he and Rous ended their collaboration, there is no evidence of an open disagreement during their collaboration. Indeed, several of the papers they coauthored imply quite the opposite, although it must be admitted that their statements therein make rather softer claims. As they concluded in one paper, for instance, "The relationship existing between the chicken sarcoma and its cause . . . seems to us to furnish some basis for the conception of an extrinsic cause for other sarcomata." In another report published a week later, they said, "No single attribute among those determined suffices to show the na-

ture of the agent; yet taken together, its characters are those we associate with micro-organisms."[17]

Based on the findings of three very different sorts of chicken tumors that seemed to share various properties, Rous and Murphy concluded that the recognition of these tumors pointed toward their causation by "a new group of entities,"[18] but, as became evident in the years that followed, the two men's ideas about the nature of this new group of entities were diametrically opposed. Rous, naturally, believed that the agent was a virus, or a minute living parasite of exogenous origins, as he made clear in his various writings and talks on the topic. Murphy equally firmly disagreed with Rous's interpretations on the nature of the sarcoma agent, as he made clear in a letter to his colleague Waro Nakahara some years after he and Rous were no longer in collaboration: "I have never believed in the virus theory [of chicken sarcoma], and that was the principle [*sic*] point of controversy between Rous and myself during the several years we worked together on the subject."[19] But there is little evidence for such a controversy elsewhere, and certainly Murphy does not seem to have openly vocalized his discontent with the virus idea during the period the two men collaborated on sarcoma work. Rous does not seem to have dwelled on the issue much either, although the difference of opinion was certainly acknowledged: "Murphy and I have always been in friendly disagreement as to what the agent is—a disagreement which may be just as well from the investigative standpoint."[20]

For his part, Rous did not pursue research on chicken sarcomas for long, moving at first to work on blood biochemistry, citing among other reasons, both the lack of any positive results or meaningful headway and the "need to broaden scientifically."[21] His bibliography reveals that he completely stopped publishing original papers on the sarcoma agent after 1915.[22] But despite later protestations—"I squirm at having the sarcoma named after me; eponyms are old hat," he once wrote to the science writer Greer Williams—he maintained a strong sense of ownership regarding his discovery, as revealed on at least two occasions.[23] In a 1929 letter to Simon Flexner in response to the former's request of Murphy—by then in charge of cancer research at the Rockefeller—for a written summary of early work on the chicken tumors, Rous protested rather "vehemently" at the choice of authorship:

> The more I think the more unendurable does the thought become. That my former assistant should, with the authorization of the Di-

> rector, summarize for our Board and for the world at large, the work for which I have lived, the real sense of the phrase, is beyond the bearing. I find it impossible to enter into an arrangement which would bring, in answer to the inevitable questionings of others, repudiation throughout the rest of my days. . . . Surely you will not ask me to yield to another my scientific identity and integrity, and not for the moment alone but for later time when medical historians will seek the *Handbuch* to learn at first source of our efforts.[24]

A couple of months later, following a lecture by Flexner, Rous wrote again objecting to his director's attribution of the discovery of the chicken tumor to both himself and Murphy. Beginning by thanking Flexner for his "delightful lecture" and generosity, Rous went on to explain his position:

> One of your statements last night raised an issue which . . . is of great importance to me. I shall discuss it, since doubtless its implications did not occur to you in the press of affairs. You said that Rous and Murphy demonstrated the existence of the filterable agent causing the chicken tumor. Now, the fact is that I carried out this work alone and published alone two papers that embodied its results. . . . Murphy had no hand in the experimental episode which showed an "infinitely little" agent to be the cause of the tumor. . . . When, now, after the lapse of more than nineteen years, you make a statement that Murphy shared in the first demonstration of the agent, you provide ground for an assumption by others that I defrauded a fellow worker in the beginning and have continued to defraud him ever since. Needless to say you would have prevented any such occurrence! But you spoke last night with authority and deliberation, leaving the impression that it had indeed occurred. The point is one so directly affecting my integrity that . . . I am unable to concede it even by keeping silent.

In the rest of the letter Rous went on to lay out a timeline of his investigations on chicken sarcoma from September 14, 1910, to January 26, 1911, where he included the dates of submission of his two single-authored papers primarily on filtration work—January 11, 1911, and February 9, 1911—as well as the date (October 1, 1910) that Murphy joined the institute and commenced work on the sarcoma project. He also offered to show Flexner the lab protocols corroborating his claims, par-

ticularly the fact that Murphy "had no share in the filtration work, even in the matter of suggestion."[25] A couple of months later Flexner responded, reassuring Rous that he would be "very circumspect to present the chicken tumor accurately. Your perturbation after my Academy lecture led me to make some discreet inquiries of just what I said about the virus. . . . It seems I made two separate statements. . . . The second one carried your and Murphy's name. I did not secure a restatement of my exact words [but] you doubtless remember them precisely."[26]

The second occasion when Rous felt compelled to defend his priority in the matter of RSV discovery occurred many decades later—by which time both Flexner and Murphy were deceased—in response to a book review by the biochemist Joseph Fruton.[27] In a manner reminiscent of his letter to Flexner, Rous began by congratulating Fruton on writing the book before going on to spell out his grievances about it.

> Your account of George Corner's history of the Rockefeller Institute has delighted him—and me well who had felt his book to be inexcusably ignored by reviewers. . . . One small inadvertent slip disturbs me: you speak of Rous and Murphy as having together found the chicken tumor virus. This carries by implication an indictment, namely that all along through the years I have ignored the rights of a fellow discoverer; never mentioning him. Actually my demonstration of the existence of a causative virus in the growth had been completed before Murphy entered my laboratory. . . . The rumor that I had been unjust to Murphy was so widely and adroitly spread soon afterwards that on learning about it I felt an imperative need to show my protocols to Simon Flexner. He deemed my evidence sound. Said protocols are still securely on file. This may seem trivial to you, so well have things gone with me since; but I greatly value your regard.[28]

Fruton was quick to acknowledge his error. "I had not known of the rumor that you had been unjust to Murphy. My esteem and affection for you would make it impossible for me to take such a rumor seriously, but if I had known of it I would have been more careful," he wrote back contritely in due haste.[29] Whereas it is not clear if Murphy knew about the entirety of Rous's exchange with Flexner on the question of priority, the fact that he harbored some proprietary feelings of his own toward the sarcoma agent, and was furthermore quite prickly on the matter of its viral identity, is evident in comments to various friends and

colleagues—both proponents and detractors—over the years. Rous, in fact, may have been the only person with whom he had no open disagreements on the topic.

In the meantime, the main reason why Rous abandoned sarcoma research so relatively early seems to have been the lack of any tangible progress for nearly four years. He made no headway in isolating observable organisms of any sort from the different types of bird tumors, which, furthermore, showed no other signs of infection in the conventional sense. He was also unable to find any examples of mammalian tumors that were transmissible via cell-free filtrate tissue. "I'd become pinched and parched mentally as a result of continually negative experimentation, and felt that only new outlooks could cure," he confided to his friend the British virologist Christopher Andrewes many years later. Moreover, he added, Flexner advised him "against publication of the negative findings, saying they would keep others from trying, who might have better luck."[30] Although Rous did eventually return to active cancer research again in the 1930s, the later work was on rabbit papillomas, not chicken sarcomas, and hence only peripherally a part of this history. So for now, I leave the chicken sarcoma, even as Rous did, to discuss the second discovery in this tale of parallels in the vicissitudes of virus research.

"AT THE EXPENSE OF BACTERIA"

It was around the time that Rous and the sarcoma virus exited the scene that the first bacteriophage made its first appearance as a relatively unremarkable player in the drama that was human disease. Soon thereafter it would also feature prominently on the broader stage of science. The first person to report the occurrence of the phenomenon we now call bacteriophagy was Frederick Twort, a medical researcher in London who was attempting to cultivate or culture "filter-passing viruses" from different possible sources in artificial media. Though unsuccessful in this regard, Twort reported "interesting results" when he attempted to culture extracts of calf vaccinia on agar: he found that the material contained a substance that had the apparent ability to dissolve bacteria called micrococci.[31] By *dissolution*, Twort meant that liquid cultures containing micrococci—normally turbid in appearance due to the growth and multiplication of these bacteria—turned clear or "glassy," when incubated with material from the vaccinia cultivations. When this material was inoculated along with bacteria to grow on on a solid medium, the normally dense and continuous "lawns" of bacteria would

be riddled with glassy patches. That the dissolution and patchiness resulted from the breakdown or destruction—called lysis—of the bacterial cells was evident from the fact that both the cleared suspension and the material from the patches contained very few or no intact bacterial cells, which were very much in evidence in samples from normal turbid cultures or bacterial lawns.

In a manner somewhat reminiscent of Rous in his first reports on the transmissible chicken sarcoma, Twort made several suggestions as to the possible cause of the observed bacterial lysis—which he would later dub "glassy transformation"—without displaying any obvious preferences.[32] "It is clear the transparent material contains an enzyme," he wrote, on the basis of his observations that the lytic substance could retain its bacteria-dissolving activity for up to six months and was destroyed by heating. Nevertheless, he conceded, "The possibility of its being an ultra-microscopic virus has not been *definitely* disproved, because we do not know for certain the nature of such a virus. . . . 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."[33]

Twort himself did not pursue this line of research any further, partly due to financial considerations and partly because he was called away soon after to serve in England's war efforts in Greece.[34] One near-contemporary, Carroll Bull, would later observe that his article "attracted little attention at the time, possibly because it appeared during the [First] World War or because of the title under which it was published."[35] In Bull's estimation the title proved detrimental because it afforded no clues that the paper was reporting the discovery of a new hitherto unidentified phenomenon. Consequently, the novelty and implications of the phenomenon of glassy transformation of the micrococci and Twort's speculations regarding their cause were not recognized for some years. References to his work did not appear until nearly a decade later.[36] When these citations did finally appear, they did so in the context of claims made by a virtually unknown scientist named Félix d'Herelle that he had discovered a new entity that he explicitly labeled as a "bacteriophage."[37]

Unlike Rous and Twort, both of whom were part of the mainstream medical research establishment, d'Herelle was a relative outsider to the scientific community. Indeed, even the details of his early life are hazy.

Based on such documentary history as his passport, wartime identity cards, and his memoirs, earlier biographers identified his birthplace as Montreal, Canada, where he was raised.[38] But in 2003 Alain Dublanchet, a French physician, found a birth certificate that places d'Herelle's birth in Paris, as Hubert Augustin Félix Haerens, the last being his mother's maiden name.[39] It was not until 1901, at the age of twenty-eight, that he appears to have settled on the name by which he is known today: Félix d'Herelle.[40] There is even doubt as to whether he received any formal education—there is some indication that he may have studied medicine, but there are no records of his graduation from any university or medical school.[41] He claimed to have taught himself microbiology, obtaining practical experience in a private laboratory that he set up in his home in Montreal in 1897.[42]

In the early twentieth century, d'Herelle gained recognition within scientific circles by working on a number of diverse problems for various scientific commissions in Latin America and North Africa, notably on pathogenic bacteria and the biological control of insect pests through these pathogens. It was during this time, according to his later autobiographical writings, that he first observed the formation of certain "glassy plaques" (*taches vierges*) on petri dishes spread with cultures of certain coccobacilli that he found to be infecting locusts and grasshoppers. But he never published anything about these findings in his copious reports of his work that he produced at the time.[43] In 1911 he became associated with the Pasteur Institutes and worked at branches in Algiers and Tunisia for some years before moving to the flagship institute in Paris. There he began to work on bacterial dysentery, which led to his discovery of bacteriophagy.[44]

The result of a bacterial infection that leads to debilitating gastrointestinal symptoms and even death, dysentery had become a matter of great urgency at the Pasteur Institute since the onset of the First World War. In 1915 there was a particularly severe outbreak in the town of Maisons-Laffitte outside Paris. The specific clinical presentation in this case had led the chief medical investigator, Georges Bertillon, to suspect that the outbreak was not caused by any of the hitherto known strains of the dysentery bacillus, and he assigned d'Herelle to investigate and manage the outbreak. In a relatively short time, d'Herelle completed his assigned task, which he described as a relatively simple undertaking:

> One did not need to be a great hygienist to find the cause: leaves had been thrown into trenches dug less than 20 meters from the kitchen, and flies, present in great numbers due to the proximity of the stables, formed a conduit between the kitchens and leaves, where we saw them placed over bloody stools. One wonders what they taught them about hygiene at the school of military medicine. I advised the filling of these trenches and digging others farther away from the kitchens, taking care to frequently treat them with chloride of lime. Once such action was taken, the epidemic was promptly contained.

Once he had resolved Bertillon's problem, d'Herelle proceeded to carry forward his own investigations using the pathological specimens from the patients. In his words:

> Several soldiers were treated at the hospital of Maisons Lafitte; I collected their stool samples for research. . . . I passed an emulsion of the dysentery stools in nutrient broth through a porcelain filter, I mixed the filtrate with a culture of dysentery bacilli and placed the whole mixture in an incubator at 37°[Celsius]; after a few hours of incubation I spread a drop of this mixture on a plate of nutrient agar and incubated it to look for the development of glassy plaques; . . . When spread on agar, on two different occasions, glassy plaques dotted the surface of the dysentery bacilli cultures. Finally I had proof that the phenomenon of glassy plaques was not limited to the coccobacilli of grasshoppers, and that they could occur just as easily in bacteria pathogenic to humans.

But what were the implications of the finding that the lytic principle of bacteria—what d'Herelle described as "le générateur"—was transmissible? By his own account, there was a period during the early phase of his experiments when he was unable to achieve any satisfactory consistency or regularity in his results working with specimens from different patients. But evidently this state of affairs soon changed, and dramatically at that, as he eloquently recalled in his memoirs:

> One day, it was in the middle of September, I was dejectedly reviewing my laboratory notebooks, I wasn't getting anywhere, when it suddenly occurred to me that it was only after I had examined the stools of the same patient multiple times, that I found the glassy

Fig 1.3 Félix d'Herelle (standing, center) with three associates working in his laboratory at the Pasteur Institute, Paris, 1919. Copyright Institut Pasteur/Archives Félix d'Hérelle.

> plaques, and even then, invariably in the final samples collected around the time just preceding convalescence. The appearance of the glassy plaques seemed to coincide with the end of the disease, and right then an idea came to me: I thought that if the "generator" of the glassy plaques, from the intestines of the grasshoppers or the dysentery patients, was the instrument of sickness, would not it also function as the instrument of healing?[45]

D'Herelle's first results were presented by Émile Roux to the French Académie des Sciences in September 1917 and published soon thereafter in *Comptes rendu de l'Académie des Sciences*.[46] There is no reference to Twort or his findings in these reports; to this day the jury is out on whether this oversight was because d'Herelle was unaware of these results or because he, as claimed later, did not think them related to his own discovery.[47] Certainly the basic phenomenon described—namely

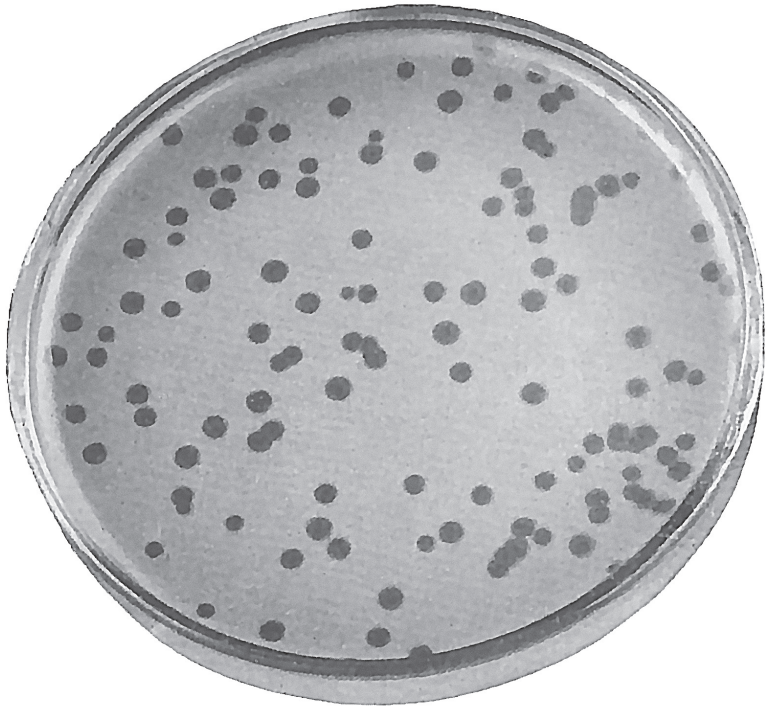

Fig 1.4 Clear plaques of bacteriophage formed on a lawn of bacteria growing in a petri dish. Photograph © 2019 by Steven M Carr, after © 1963 by WH Freeman.

that of a transmissible bacterial lysis—was the same. But the investigative contexts and interpretations in the two cases were completely different.

Twort, as previously discussed, had come across the phenomenon in a search for ways to cultivate viruses. He was primarily concerned with the phenomenon as it appeared in micrococci, although he had extended his studies to other material and had devoted the last couple of paragraphs to describing a "dissolving substance [of] bacilli of the typhoid-coli group" on which he hoped to continue studies at a later date.[48] But where Twort had suggested multiple possible explanations for the phenomenon, d'Herelle, from his very first paper, interpreted his findings only one way and was unequivocal in his statements that they were viruses. True, he used the the phrase "microbe invisible" in his French publications, but he used *virus* in his earliest English language writings on the bacteriophages. In a discussion of various hypotheses about source of the substance that caused the lysis of bacte-

ria, for instance, he suggested that they could be "secreted by an ultramicroscopic virus, which is a parasite of bacteria. This is the hypothesis by which I have held since my first publication."[49]

As for the term *bacteriophage* itself, which d'Herelle introduced in his first paper, it does not seem to have been originally intended as a neologism for a brand-new discovery. He began his paper with the declaration: "From the feces of several patients convalescing from infection with the dysentery bacillus, . . . I have isolated an invisible microbe endowed with an antagonistic property against the bacillus," and in his conclusion even gave this putative agent of lysis a name: "This microbe, the true microbe of immunity, is an obligatory *bacteriophage*."[50] He appears to have used the term in a descriptive sense, as something that lived at the expense of these bacteria in much the same way as the bacteria themselves lived at the expense of their human hosts. As elaborated in his monograph, which d'Herelle published within a few years of his initial discoveries, "The suffix 'phage' is not used in its strict sense of 'to eat' but in that of 'developing at the expense of;' a sense that is frequently used elsewhere in scientific terminology. . . . This is precisely the interpretation to be given the term 'phage' in the word 'bacteriophage.'"[51]

Although at first it seemed as though d'Herelle and his discovery would fade into obscurity like Twort, the same war that had stalled or stopped Twort proved to be a catalyst for d'Herelle.[52] After a short lull of about two years, d'Herelle's bacteriophage began to garner an ever-widening interest from the scientific community, beginning with scientists who "geographically closest to him" and rippling outward to North America and even Australia.[53] As bacteriophage researcher Donna Duckworth has pointed out, in those first few years, "Hundreds of people cited d'Herelle's work, and although he may not have been universally regarded, he was certainly universally acknowledged."

One possible reason why d'Herelle's work drew more attention than Twort's discoveries is that in addition to claiming novelty he emphasized the medical implications of his findings, not only for understanding infectious diseases and immunity but also for disease therapy. As Duckworth noted, "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."[54] Indeed, the potential applications of the antagonist as an immu-

nizing or therapeutic agent against dysentery was to remain at the forefront of d'Herelle's interests for many years to come.

Twort, on the other hand, had made no connections between transmissible autolysis and immunity or antimicrobial therapy in his paper. Also, after returning from the war, he seems to have returned to his search for ways to culture viruses rather than pursue investigations into the nature of glassy transformation.[55] In fact, Twort appears to have remained as unaware of d'Herelle's initial work as the latter maintained he had been of Twort's 1915 discoveries, for he made no public comments about it until his own work was brought into the spotlight by the microbiologists Jules Bordet and Mihai Ciuca (as detailed in chapter 2).[56]

On a few occasions thereafter, however, Twort would vigorously defend the claim that this group had staked on his behalf. "May I point out that that the work of d'Herelle is little more than confirmation of my work," he wrote to the *Lancet* in 1921.[57] And upon reading a review of Sinclair Lewis's Pulitzer Prize–winning novel, *Arrowsmith*, in which the bacteriophage was a major plot device, he sent a similar note again, objecting that "the author gives the credit for the discovery of the phenomenon of bacterial lysis caused by a contagious filter-passing material to Dr. d'Herelle of the Pasteur Institute." Furthermore, he added: "At the time of the publication of the paper I was asked to undertake duties in the army in Salonika, and I had no opportunity to work out any additional details connected with the phenomenon, although when in Salonika the whole subject was discussed with the Canadian, French, and British medical officers there. The first work of d'Herelle on the 'lysin,' named by him the 'bacteriophage,' associated with the dysentery bacilli was not published until nearly two years after the appearance of my paper."[58] The editors of the *Lancet* seem to have had only limited sympathy for Twort's claim, responding to it with the terse footnote, "We have referred to the matter in our columns as the Twort-d'Herelle phenomenon on more than one occasion."

This note from the editors serves to illustrate the impact that the participation of a famous personage can have on a scientific debate, for before Bordet—the sole recipient of the 1919 Nobel Prize—entered the bacteriophage fray, scientists had used d'Herelle's label of *bacteriophagy* to describe transmissible lysis quite unproblematically. After the priority issue was raised, however, the phenomenon came to be called the Twort-d'Herelle phenomenon for a time. But in the long run it was the shorter label that stuck, and remains in use to this day.

The incident also exemplifies, once more, the value of a comparative Plutarchian narrative in showcasing the small details and nuances of an episode that may have been overlooked in individual, perhaps more linear histories. Both in the case of bacteriophagy and in that of the discovery of the chicken sarcoma agent, for instance, the priority issue was spurred by people not directly involved in the actual investigations: Flexner and Fruton, as shown in the instance of Rous, and Bordet's lab in the case of d'Herelle. But Rous was only charged of wrongdoing by implication—and inadvertently at that—by both Flexner and Fruton, both of whom acknowledged their errors. Bordet and his colleagues, on the other hand, claimed that Twort's work had been overlooked by d'Herelle, albeit unknowingly, and roundly declared that they believed it was "a duty to recognize the incontestable priority of Twort in the study of this question." In this paper, which was first presented before the Belgian Society for Biology in March 1921, the authors also announced, "The burden of an exact history makes it necessary for us to cite a previous work which d'Herelle has not known and that we ourselves have been ignorant of until now that contains the observations that d'Herelle had 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."[59] In contrast to Rous, who had expressed his concerns in private letters to Flexner and Fruton, d'Herelle defended his position publicly many times. The first occasion was at a meeting of the Society for Biology in Paris—the French counterpart to the society where Bordet and Ciuca had earlier presented their papers—and his presentation was later published in the Society's proceedings.[60] In this and other early response to his critics, d'Herelle neither denied nor admitted to having prior knowledge of Twort's 1915 work; rather, he emphasized the difference in their findings, arguing that Twort's description of the phenomenon with micrococci was "not a question of a real bacterial dissolution, but a transformation of a normal culture on agar into a glassy and transparent one."[61]

For reasons that are not entirely clear, d'Herelle "abruptly left" the Pasteur Institute in Paris in 1921 and over the next two decades or so pursued his studies on bacteriophages, especially their use in therapy against bacterial diseases, in various places, including Leiden, the United States, Egypt, India, and Russia.[62] His career trajectory—the pursuit of a single topic in many far-flung places—presents a striking contrast to that of Rous, who pursued many different topics over the course of his career, but stayed at the Rockefeller throughout. What both men

shared, however, was a steadfast belief in their interpretation of their findings and an active participation in debates over the viral identity of their respective discoveries for many decades.

WHY VIRUSES?

Why did both Rous and d'Herelle think of their discoveries as "viruses"? How exactly did they conceptualize the entities? The answers to these questions are by no means straightforward, for the term *virus* represents a very good example of a scientific concept that has undergone multiple changes, or "variances," in meaning over time, to borrow the vocabulary of the historian of science and medicine Ilana Löwy.[63] It was only in the 1950s that *virus* came to acquire the definition we recognize today: an obligate intracellular parasite basically composed of a single type of nucleic acid encased in a proteinaceous coat.[64] A survey of earlier literature shows widely varying definitions for the term for at least half a century prior: "Every virus is a microbe," the famed Louis Pasteur had declared in 1890, whereas just over two decades later, the Harvard bacteriologist S. B. Wolbach was defining filterable viruses far more specifically as "microorganisms which will pass through filters, the pores of which are too small to give passage to ordinary bacteria."[65] As Löwy argued: "Scientists naturally employ the vocabulary of their discipline. But we should not assume that the meaning of the terms remain constant. . . . Within a single scientific community at least, scientists assume that a given term has the same meaning for every potential reader of a scientific work. [They] employ terms to sum up a scientific consensus at a given moment, and, more importantly, they assume that a consensus does indeed exist. Many scientific terms do not, however, possess a single, well-defined meaning."[66]

Rous retrospectively alluded to such a fluidity and change in the meaning of *virus* when discussing the reasons he had refrained from using the term in his early publications: "I wanted to call the tumor cause a virus, but the crusty, redoubtable, lovable old Secretary of the Board of Scientific Directors, Dr. [T. Mitchell] Prudden, whose wisdom I admired, put his granite foot down against it, suggesting 'agent' instead."[67] He also admitted that by not letting him use this term in publications, the "older and wiser" Prudden had done him "a good turn, since the virus proved in some ways so peculiar that not until the time of my Harvey lecture . . . , when the traits of viruses generally were better realized[,] could it safely be called as such."[68]

Given the intellectual and institutional contexts of Rous's discov-

ery, it is actually rather remarkable that he considered viruses or any sort of living parasite as a possible cause of the chicken sarcoma at all. His colleague Murphy's attitude, discussed in greater detail in later chapters, was rather more typical of what one might have expected from someone trained in medical pathology, especially in the immediate wake of a consensus—arrived at during an international cancer congress in 1910—that cancer and tumors could not be caused by living organisms or parasites.[69] Based on his body of work at the time of the discovery, it is possible to discern that Rous's definition of a virus at the time of his initial discovery of the sarcoma agent in 1911 was that of an extremely tiny *living* infectious organism that was invisible under a light microscope and could pass through bacteriological filters impermeable to the smallest known bacteria.

Rous's willingness to accept that a tumor could be caused by such an entity indicates an extraordinarily flexible mind, but one must also consider the influence of his work environment in shaping his ideas. Simon Flexner, who had hired Rous directly and principally to work on the cancer problem, had done so within his own division of pathology and *bacteriology* at the Rockefeller. In part such a move was due to the fact that Rockefeller had not prioritized cancer research at the time of its founding—reflecting the aforementioned attitudes of such prominent medical researchers as William Welch, who was also the president of the institute's board of scientific directors.[70] Nevertheless, as Rous and others experienced firsthand, it provided an environment that was particularly conducive to new ideas.[71] Flexner, who as founding director of the institute would have has a greater degree of involvement with its day-to-day affairs, compared to Welch, was not as pessimistic about the prospects of this field. In fact, he had led the way at the institute with his 1906 discovery of a transplantable tumor of rats.[72] Although he never worked on the avian sarcoma problem himself, he remained in Rous's corner, so to speak, on the matter of the possible viral etiology of the tumor.[73]

Of the various criteria, it was the living nature of the sarcoma agent that was perhaps the most difficult for Rous to demonstrate. Then, as indeed is the case even today, the "most direct means of proving that the agent is alive is to grow and transfer it in culture," as Rous and Murphy rightly pointed out in their report.[74] But the recognition that viruses are fundamentally different than bacteria and thus would require rather different materials and conditions for growing in culture, was many years away. Consequently when Rous tried different ways to

cultivate—that is to say, grow and propagate—the sarcoma agent in vitro, he did not succeed. Meanwhile, his reason for thinking that the sarcoma agent might be living was based on numerous pieces of indirect evidence, including its survival—the retention of its biological activity under different conditions known to destroy or inactivate other living cells—and its capacity to multiply in tissues of unaffected birds when injected therein.[75] In one of their earliest joint papers, Rous and Murphy had concluded, "No single attribute among those determined suffices to show the nature of the agent; yet taken together, its character are those we associate with micro-organisms."[76] In a single-authored publication later that year, Rous cited more specifics regarding the sarcoma agent's behavior under different physical and chemical treatments, and concluded even more definitively: "The various features seem sufficient to identify it as a living organism in distinction from a ferment."[77]

D'Herelle, as seen, had showed not the slightest bit of hesitation in declaring his find "an invisible microbe," or virus, in both the title and opening sentence of his very first presentation about the transmissible agent of bacterial lysis isolated from the stools of dysentery patients. Relying on much the same line of reasoning as Rous had for the sarcoma agent—namely, its capacity to multiply in fresh uninfected host cells—he also went on to offer what he believed was "visible evidence" for the lysis being caused by a living agent: "If one adds to a culture of Shiga [the dysentery bacillus] approximately one to a million of an already lysed culture, and if, immediately after, one spreads out on an agar slant a drop of this culture, one obtains, after incubation, a coat of dysentery bacilli showing a certain number of circles about 1 mm in diameter, where the culture is void; these points can only represent the colonies of the antagonistic microbe: a chemical substance would not be able to concentrate at defined points."[78] A few years later, he elaborated his argument with the description of an experiment and the following interpretation of its results:

> It is the presence of these immutable bare spaces, which are perfectly circular, that characterizes what we have named "bacteriophage." . . . The number of spaces depends simply on the quantity of filtrate added to the bacterial culture. If into various bacterial emulsions we introduce variable quantities of the filtrate, the number of bare spaces is strictly proportional to the quantity of filtrate added. On the other hand, the number of the bare spaces is inde-

> pendent of the number of bacteria contained in the medium. . . . The phenomenon of these vacant spaces is only comprehensible on the supposition that the bacteriophagic principle . . . the source of the lytic enzymes, is a corpuscle; and that each corpuscle deposited on the agar in the midst of the bacteria gives rise to a colony of these ultramicroscopic corpuscles, such a colony being represented by a bare space.[79]

In the same paper, d'Herelle added, unconsciously, and almost uncannily echoing Rous in both the nature of evidence and reasoning, "The behaviour of bacteriophage towards physical and chemical reagents is that of a living being, and does not agree with that of an enzyme." He held tenaciously to this original conception throughout his life, defending his position against challenges and attacks from different fronts and for many years. What is perhaps his most detailed treatment of this issue appeared in his second monograph on bacteriophages, the English translation of which was advertised using d'Herelle's own words: "Of the present book the author says, 'I offer physiological proof of the living nature of the bacteriophage, an infravisible parasite of bacteria. Logic demands that the evidence which I have provided be justly evaluated before contrary theories be affirmed.'"[80]

Despite the differences in the particulars—of geography, investigative goals, experimental systems, and even of specific terminology that they used—then, Rous and d'Herelle had a fundamental idea in common, one that would stay with them through the decades that followed. Both conceived of their discoveries as "infective agents" of some extrinsic or exogenous origin, which caused their effects by entering, or infecting, the host cells, and somehow disrupting normal functions within—in other words, as viruses.[81]