Introduction: A Short History of Virology

Abstract
Viral infections have been recorded unknowingly from the beginning of recorded history. The ancient Greeks and Romans described plagues of unknown origin. In general, infections were blamed on sins and punishment, balances of “vital humors” or on “miasma,” (rotten smells). In the sixteenth century, Girolamo Fracastoro suggested that infectious agents might spread disease, as did Agostino Bassi, studying diseases of silkworms. The development of the microscope by Hook and Van Leeuwenhoek in the seventeenth century led to the discovery of a new living world inhabited by very small creatures. Edward Jenner in England demonstrated that smallpox, a dreaded disease, could be prevented by inoculation with an organism that caused pockmarks on cows and dairymaids; this was the beginning of the concept of vaccination. Louis Pasteur proved that fermentation only occurred in the presence of air and was due to microorganisms. Pasteur and Pierre Roux, a colleague, as well as Jacob Henle and Robert Koch, proved that germs caused bacterial diseases such as anthrax and tuberculosis; Pasteur and Roux developed a vaccine against rabies by passaging the infectious material through rabbits. By the end of the nineteenth century it had been established that most infectious diseases were the result of germs. In parallel with this research, plant scientists had isolated material that passed through a low pore filter that was infectious to tobacco plants. This was called a “virus,” from the Latin for poison. Viruses were also found to be associated with leukemia and other cancers of chickens. The twentieth century saw the discovery of bacteriophage, viruses that attack bacteria, and the use of such bacteriophage to launch studies of molecular biology, and DNA and RNA structure.
1.1 What Is a Virus?

Although historians identified pandemic diseases, which spread around the world, the agents responsible for these were not recognized until the early and mid-twentieth century. Some of these pandemics, such as the Black Death, were caused by bacteria, and others by viruses such as smallpox. The word virus comes from the Latin meaning poison or slimy matter, and entered the English language to signify infectious disease.

Even today there is discussion as to whether viruses are “living” entities. Viruses are so small that they are invisible even at high magnification with a light microscope. Viruses cannot be grown independently in culture as can bacteria and other free-living microorganisms; viruses require a living cell for reproduction, whether animal, bacterial or plant. A virus contains genetic information—DNA or RNA—but the statement that a virus is alive is a controversial one. In the 1930s, Wendell Stanley was able to form crystals in solution of the tobacco mosaic virus, a feat that had been performed up until then only with pure chemical molecules infinitesimally smaller than a virus. Viruses form aggregates (clumping) in solution and bond chemically with each other to form a crystal. Many different viruses have been crystallized, and their basic shape and composition revealed. Viruses are at the edge of life, having attributes of inert objects, such as the ability to form crystals, yet also possessing characteristics associated with life, such as genetic material and reproduction. One virus can replicate and give rise to millions more, which is certainly an attribute of life. A virus exists in two stages, one as an inert chemical with a chemical formula; for example, the chemical formula for poliovirus is C332,652 H492,388 N98,245 O131,196 P7,501 S2,340 [1]; when the virus enters a living cell, it becomes an active replicating organism.

Viruses are genetic parasites—that is, they require the protein synthetic machinery of the host to reproduce. Viruses, as visualized by the electron microscope, are symmetrical and complicated structures. Modern-day computers and electron microscopy allow us to produce fantastic pictures of viruses (Figs. 1.1, 1.2).

1.2 Plagues Before the Germ Theory

The concept of “germs”—something invisible that can cause illness—is a quite recent one. The germ theory is in reality a product of the nineteenth century; until then, disease was thought of as either a divine intervention and punishment, or the result of “miasmas” (bad air, stench of rotting material), or on the balance of four vital humors: blood, phlegm, black bile, or yellow bile. The theory of “in balance in humors” (body fluids) dates back to the ancient Greeks or even earlier. Currently, some individuals, and even whole societies, believe that AIDS and other diseases are the result of divine punishment for specific lifestyles. The idea of miasmas being responsible for disease lasted until the early nineteenth century.
Fig. 1.1 Electron micrograph of hepatitis B virus capsid (courtesy of Adam Zlotnik)

Fig. 1.2 Artist’s rendition of adenovirus (Cronodon.com, with permission)
In the eighteenth century, the smell of rotting coffee beans was considered the causative agent by some, as an explanation for the Yellow Fever epidemic in Philadelphia.

**Plagues** have been recorded since the beginning of written history. Certainly the Bible discusses hygienic precautions against leprosy, the isolation of lepers (a kind of quarantine) and the precautions to be taken against infection by lepers. Two of the biblical plagues of Egypt may have been bubonic plague (boils), and rinderpest of cattle, still prevalent in parts of Africa today. The ancient Greeks and Romans recorded many plagues. Thucydides recounts the great plague that swept through Athens during the Peloponnesian Wars in 430 B.C.: “The dead lay unburied, the temples were full of corpses. Dying wretches were gathered at every fountain, seeking to relieve their unquenchable thirst.” Thucydides writes that the plague began in Ethiopia, spread throughout Egypt, over the Persian Empire, and then reached Athens.

There are reports of a similar plague in Rome at about the same time. From Thucydides’s description of the symptoms, it is difficult to discern whether this was smallpox or measles in a very virulent form or, as has been suggested, some bacterial infection such as salmonella typhimurium, or Yesinia pestis, the black death. He describes the onset of fever, sneezing and coughing, with the discharge of blood and a fetid smell, followed by the appearance of ulcers and small pustules on the skin. This was followed by diarrhea and death. Thucydides claims that birds of prey that devoured the bodies also died, and that the disease could spread to domestic animals. His description of the effect on physicians and others who nursed the sick is similar to our descriptions today of outbreaks of dreaded diseases such as hemorrhagic fevers, Ebola and dengue.

Thucydides also writes that those who recovered from the disease were rarely affected a second time, thus indicating a recognition of immunity. In 542 A.D., the plague known as the “Plague of Justinian” hit Rome. The description would suggest that this was the bubonic plague (Black death—*Yersinia pestis*), a bacterial disease that periodically swept through Europe in the late Middle Ages and decimated one-third of the population of Europe.

Apart from the bubonic plague, the other great plagues affecting man were smallpox, and, later on, yellow fever—both of viral origin. With the Europeans’ colonization of the Americas, viral plagues were important in shaping the history of the continent. Whole populations of native peoples of North and South America were wiped out by smallpox, and, to a lesser extent, by measles. When Cortes landed in Mexico in 1519, the population of Mexico was estimated to be between 25–50 million. Fifty years later the population was only 3 million. Most of the ravages were due to diseases to which Europeans were semi-immune, not the superior forces of the Spaniards.

The fact that viruses have been afflicting humans since the beginning of written history is indubitable. Pockmarks similar to those of smallpox have been found on the face of a mummified Pharaoh recovered from the pyramids of Egypt. Some drawings on the tombs from the same period indicate wasting of limbs that could be due to poliovirus. It has been suggested that viral infection of humans occurred as a result of the transition from a hunter-gatherer society to a settled agricultural society.
with the domestication of other mammalian species and the transfer of viruses from these animals to humans. The primitive hunter may have been a healthier person than the settled “gatherer,” since he lived a more isolated life, without any crowding. The proximity to domesticated animals and groups of people living under one roof probably resulted in the introduction of new infections to the human population. Examples of this have occurred with human infections in the last few years, with the spread of avian flu, SARS, and HIV—all from other animals.

1.3 Development of the Microscope and Germ Theory

The development of a simple light microscope in the mid-seventeenth century by Robert Hooke (1635–1703) in England and Antonie van Leeuwenhoek (1632–1723) in Holland gave rise to the identification of “animalcules,” microorganisms of various shapes, such as bacterial bacilli (rods) and cocci (spheres), and even spirochetes (twisted structures with flagella—tail-like appendages) reviewed in [2]. Hook described the basic cell, using slices of cork under his crude microscope. Leeuwenhoek explored the contents of many substances, including rainwater collected in barrels, flora from the mouth, semen, etc. He described tiny organisms using his microscopes that could magnify up to 300 times. These observations opened up a previously invisible world to science and allowed advances in many areas beyond diseases. An entire invisible world existed but it did not, however, include viruses, which are too small to be seen even with the best light microscope (Figs. 1.3, 1.4).

The first person to really describe diseases as caused by infectious agents was an Italian physician, Girolamo Fracastoro (1478–1553) (Fig. 1.5). He was an acute observer of the plague in Verona and he published many books on syphilis, a new disease of the time, as well as the basis of its contagion. Not only was he a physician, he was also a poet. The name “syphilis” is derived from Fracastoro’s 1530 poem in three books, *Syphilis sive morbus gallicus* (“Syphilis or The French Disease”), about a shepherd named Syphilis, who angered the god Apollo and was thus cursed with this disease. ([http://en.wikipedia.org/wiki/Girolamo_Fracastoro](http://en.wikipedia.org/wiki/Girolamo_Fracastoro)). The poem goes on to describe the symptoms of the disease.

A shepherd once (distrust not ancient fame)
Possest these Downs,
and Syphilus his Name;
Some destin’d Head t’attone the Crimes of all,
On Syphilus the dreadful Lot did fall.
Through what adventures this unknown Disease
So lately did astonisht Europe seize,
Through Asian coasts and Libyan Cities ran,
And from what Seeds the Malady began,
Took from France his name.
Our Song shall tell: to Naples first it came
From France, and justly named.

1.2 Plagues Before the Germ Theory
Fig. 1.3  The Leeuwenhoek microscope

Fig. 1.4  Drawings of protozoa and other “animalcules” from Leuwenhoek (image from Clendening Library, U. Kansas Medical Center)

Fig. 1.5  Portrait of Girolamo Fracastoro by Titian, National Gallery, London, U.K. painted 1528
Fracastoro discussed the spread of disease that resulted from touching contaminated clothing (fomites) as well as from contact among individuals. He also discussed how various diseases infected and affected various age groups in a population and in various ways. Syphilis was later shown to be a bacterial disease and was called the “great pox,” as opposed to smallpox, the virus disease, although smallpox was the more deadly of the two [3].

Another pioneer of the germ theory was Agostino Bassi (1773–1856), an Italian lawyer and later agriculturist. He devoted a large part of his life to studying muscardine, a disease of the silkworm. Muscardine was an important fungal pest of the economically important silk industry of Northern Italy. Many farms were abandoned because of this disease. Bassi concluded from his studies that an infectious agent that could be transmitted by contact between worms, and also could be transmitted by contaminated cages or utensils, caused the disease. He identified the infectious agent as a fungus, later named \textit{Beauvaria bassiana}. He is also credited with rescuing the vitally important silk industry with recommendations such as the use of disinfectants; separating the rows of feeding caterpillars; isolating and destroying infected caterpillars; and keeping the silk farms clean. He used “semi-sterile” techniques to prevent infection. Pasteur and others who came after him were unaware of his research and made similar, independent discoveries. Bassi also proposed that human diseases such as syphilis and measles were transmitted from one person to another by contact. Both Fracastoro and Bassi have been overlooked in the history of medicine, whereas Pasteur has become a household name [2].

1.4 Vaccination in the Time of Jenner

Vaccine development began with attempts to prevent smallpox infections using variolation (extracting pus from smallpox lesions) and rubbing the pus onto the arm or leg of an uninfected person. Variolation was first performed in China and later in the Middle East in the fifteenth and sixteenth centuries. The practice of variolation was introduced into England by Lady Mary Montagu, wife of the British ambassador to Turkey. She had discovered the use of variolation during her sojourn in Turkey in 1716–1718; variolation was widely practiced, but often resulted in cases of smallpox, although not as severe as a natural infection.

Dr. Edward Jenner (1749–1823), a practicing physician in Berkeley, England, had noted, as had others, that milkmaids very rarely contracted smallpox. Instead, their hands often had sores later shown to be caused by the cowpox virus (vaccinia) (Fig. 1.6). Jenner had a small laboratory not far from Berkeley Castle in northern England, where he did experiments.

Jenner was not the first to recognize the connection between cowpox and immunity to smallpox. A number of farmers had previously made this observation. For example, Benjamin Jesty, a farmer in Dorset (southern England), had inoculated his wife and children with cowpox material during an outbreak in
1774 and, I assume, induced immunity in the family. In 1796 Jenner tested his hypothesis that the pus from the wounds that occurred on the udder of milking cows contained material that could protect against smallpox by inoculating James Phipps, the son of his gardener, with pus from a wound on the hand of his milkmaid, Sarah Nelmes, who had been infected by a virus from a cow named Blossom. Blossom is probably one of the most famous cows in history, her hide immortalized in St George’s Hospital Medical School in London. James was then variolated with the smallpox virus a number of times and had basically no reaction, showing complete immunity. Jenner then tested vaccination on 23 other people, none of whom came down with smallpox. He presented his data to the Royal Society and published his data [4]. His idea of preventing smallpox with a “vaccine” was not easily accepted on religious grounds (“interfering with the work of God”); also ridiculed was the use of material from a cow (see cartoon, Fig. 1.7) (see Chap. 19, Vaccines).

In this cartoon, the British satirist James Gillray drew a caricature of a scene at the Smallpox and Inoculation Hospital at St Pancras, showing Edward Jenner administering cowpox vaccine to frightened young women, and cows emerging from different parts of people’s bodies. The cartoon was inspired by the controversy over inoculating against the dreaded disease—smallpox. The inoculation agent, cowpox vaccine, was rumored to have the ability to sprout cow-like appendages. A serene Edward Jenner stands amid the crowd. A boy next to him holds a container labeled “VACCINE POCK hot from ye COW”; papers in the boy’s pocket are labeled “Benefits of the Vaccine.” The tub on the desk next to Jenner is labeled “OPENING MIXTURE,” and bottle next to the tub is labeled “VOMIT.” The painting on the wall depicts worshippers of the Golden Calf. (from Wikipedia).

Around 1800, Dr. J. Clinch, a medical missionary in Newfoundland, began using Jenner’s vaccine and by the end of 1801 he had vaccinated approximately 700 people. This was probably the first vaccination of an entire community in North America. Jenner died in 1823 as the result of a stroke. The British government passed the vaccination act in 1840, making variolation illegal and supplying smallpox vaccine (cowpox) free of charge. In 1853 the vaccination act
was passed, requiring all children to receive smallpox vaccines by the age of 4 months. Later on, the act was changed to allow parents who objected to vaccination to appeal. In 1905 the U.S. Supreme Court ruled that states could impose mandatory vaccination on children.

Smallpox was declared eradicated from the world in 1980, after an intensive challenge to stamp it out. In fact, eradication really began in 1803 when the Spanish crown attempted to vaccinate the entire population of the Philippines. In 1813 the U.S. Congress passed the vaccination act to insure safe vaccines for the U.S. public. By 1832 the U.S. government had set up a program for the vaccination of American Indians; known as the Indian Vaccination Act. Unfortunately it did not eradicate the spread of the virus and was misused to remove Indians to reservations. Thus there was a piecemeal attempt to eradicate the virus in a few areas of the world, such as India and the East Indies. By 1897 smallpox had been eradicated from the US and by the beginning of the twentieth century from most of the industrialized countries of Europe. Despite this, 2,000,000 people died annually from the disease in the 1950s; therefore, a campaign was launched in 1959 to completely eradicate the virus from the hemisphere. Although it took longer than expected, the last cases of smallpox occurred in Somalia in 1977 [5, 6].

### 1.5 Louis Pasteur and Robert Koch

The two giants of microbiology who changed the history of medicine and led to “modern” clinical medicine were Louis Pasteur (1822–1895) in Paris, and Robert Koch (1843–1910) in Berlin. In 1857–1858 Pasteur made the discovery that put to
rest the theory of spontaneous generation of life. He showed that liquids in sealed containers remained sterile, but that exposure to the air resulted in contaminations that gave rise to fermentations or growth of bacteria or fungi. He wrote, “germs of microscopic organisms abound in the surface of all objects, in the air, and in water,” concluding that these organisms cause fermentation, such as occur in wine, beer, vinegar etc., and that under sterile conditions with no access to air these fermentations do not take place.

About the same time, working in Germany, Robert Koch, a country doctor, described the organism responsible for anthrax, a disease of cattle. He showed that the bacteria had a life cycle, including a stage of spore formation, a stage not easily destroyed by heat or other means, which could account for infections occurring at various periods, and allowed organisms to live in the soil for a long time. Among other achievements, Koch went on to isolate and describe the organism responsible for tuberculosis. Koch was influenced by his mentor, Jacob Henle, who in 1840 proposed that infectious diseases were caused by living organisms and reproduced outside the infected individual. Earlier, Aloys Pollender, Pierre-Francois Olive Rayer and Casamir-Joseph Davaine had discovered the anthrax bacterium. Koch himself set out to prove scientifically that this bacillus is, in fact, the cause of the disease. He inoculated mice with homemade slivers of wood containing anthrax bacilli taken from the spleens of farm animals that had died of the disease, and found that these mice were susceptible to the disease and died, whereas mice inoculated at the same time with blood from the spleens of healthy animals did not. This confirmed the work of others who had shown that the disease could be transmitted by means of the blood of infected animals. He went on to develop methods of growing and staining the bacteria, and with a colleague developed the Petri dish (named after his colleague) that is still used today [7].

Koch also isolated the bacterium responsible for tuberculosis, and from this developed specific postulates. To prove the causative role of the bacillus...

...the bacilli had to be isolated from the body and cultivated in pure culture until devoid of all adherent products of disease originating from the animal organism; and, finally, through transfer of the isolated bacilli to animals, the same clinical picture of tuberculosis as is obtained empirically by the injection of naturally developed tuberculous material had to be produced.

Koch or Emile Roux (1853–1933) proposed this simplification of his famous postulates:

An organism must be isolated from the diseased animal.
It must be possible to grow to a pure culture.
It must be possible to re-infect the animal.
It should be possible to re-isolate the organism.

These postulates are still used as the basis for characterizing an infectious agent, but have to be modified in that it is important to emphasize that the disease produced has to be similar to the one from which the material is taken. For example, in using an animal model system, such as the mouse, the disease produced may not be identical in that animal to the one produced in humans. It is also...
important that the bacteria or virus be found in all cases of the particular disease. As will be discussed later, in the case of work with yellow fever, bacteria were isolated from most yellow fever victims (not all, however) and was claimed to be the source of the disease. Only by showing that it was not present in ALL patients could it be proven that it was not the causative agent. It has proven ethically difficult to carry out Koch’s postulates in the case of some viral diseases that only infect humans (e.g., human immunodeficiency virus [HIV] and hepatitis C). If no animal model is present, it is difficult to prove beyond a doubt—without human experimentation—that a specific agent is the cause of the disease. Smallpox and measles viruses also are exclusively human pathogens, and the early study of smallpox in particular involved unregulated research on human subjects. In more recent times, and particularly in the era of laboratory-driven investigations, we have had to create very specific ethical guidelines. Many of the discoveries, such as the role of mosquitoes in transmitting yellow fever, and vaccination against smallpox, involved the use of humans in a manner not acceptable today.

In 1878, in a paper read before the French Academy of Sciences, Pasteur presented evidence that disease was caused by discrete germs. He demonstrated this for the anthrax bacterium (*Bacillus anthrax*) isolated as described above by Koch. Furthermore, he showed that minimal numbers of the bacteria are required by serial dilution, growing the new cultures in test tubes and injecting material into animals (see Koch’s postulates above). Anthrax today is better known as a possible agent of biological warfare than as a disease of cattle. In 2001, envelopes containing anthrax were sent through the U.S. mail to congressmen and caused quite a panic. It was initially assumed that this was a case of bio-terrorism conducted by foreign terrorists, although now it is suspected that this was the work of a disturbed American scientist.

Pasteur also successfully isolated organisms involved in sepsis, which results in septic shock due to the growth of bacteria and resulting toxins in the blood. During this study he demonstrated differences between anaerobic and aerobic organisms and identified bacterial spores that could give rise to septicemia (blood poisoning). Such spores survive for a long time outside the body. Koch had made a similar finding—that anthrax could exist in a spore form resistant to heat and desiccation. In a paper in 1880, Pasteur described the application of his findings and techniques to other conditions, particularly furuncles (boils). He showed that the pus that developed from such lesions contained infectious chains made of round cells (cocci) and that this organism could also be grown in culture. He showed that every furuncle contained cocci that caused the inflammation and pus formation. These organisms were injected into rabbits and formed abscesses containing pus, and the same organism could be isolated again (Koch’s postulate). Of great interest were his observations on puerperal fever, associated with women who had recently given birth or had a miscarriage, and which was extremely fatal, and the isolation of the bacteria that caused this disease [8] (Figs. 1.8, 1.9).

Ignaz Semmelweis (1818–1865) (Fig. 1.10), working independently in Vienna, had shown that puerperal fever could be prevented by handwashing in a chlorine-lime solution and that it was often spread by physicians after autopsy, or following
treatment of sick women. Semmelweis was ridiculed for suggesting that doctors, midwives or nurses spread it. Handwashing was not a common practice, and Semmelweis insulted the egos of the medical profession by accusing them of spreading the bacteria. His ideas were accepted only after his death, which occurred in an asylum at the age of 47. Only a few weeks after being confined to the asylum, his guards beat him to death. [8, 9].

It could be shown that the organism causing puerperal fever could be grown in rabbits. In his paper of 1880, Pasteur also proposed the use of antiseptics to kill the infecting organisms. He proposed the use of boric acid rather than the carbolic acid then in use. Boric acid was used in the treatment of urinary tract infections by injection into the bladder. He also proposed placing a pad soaked in boric acid at the bedside of each woman during confinement to prevent puerperal fever. This infection was shown to be caused by streptococci and today is treatable with antibiotics (Fig. 1.10).
1.6 How Can We Differentiate a Virus from a Bacterium?

The differentiation of viruses from other types of disease-causing organisms was initially based on size and filterability. Viruses are 100–1,000 times smaller than bacteria, and this could be determined only when one began to filter cultures from infected individuals, plants or animals. Various types of filters were invented in the nineteenth and early twentieth centuries to purify water and other liquids. These early filters were made of diatomaceous earth from organisms that left a glass shell after they died, and small matter would pass through the filters, but larger organisms, such as most bacteria, fungi, etc., would be retained by the filter. Thus viruses could be defined as “filterable agents.” These porcelain filters were developed in the Pasteur group by one of Pasteur’s colleagues, Charles Chamberland (1858–1901), and were called “Chamberland filters.”

However, it was difficult to differentiate “viruses” from toxins. Work in the lab of Louis Pasteur, performed by his colleagues Emile Roux and Alexander Yersin (1863–1943), identified the toxin that caused diphtheria. Roux and Yersin developed an anti-serum to this toxin, which was used to save the lives of many young adults affected by diphtheria, a fatal disease. Filtration was an important tool in differentiating bacteria that had been retained by the filters from toxins and what were later called viruses. Diphtheria toxin is actually produced by a strain of bacillus containing a bacteriophage (virus). The toxin is produced by the virus.

Until very recently, the concept of a virus being a filterable agent, and much smaller than bacteria, was an accepted dogma of virology. However, within the last few years, viruses that parasitize protozoa and other lower forms of life have been isolated; they are as large as bacteria and contain considerable genetic material. Thus the idea that viruses are filterable, small and contain a small number of genes, is no longer tenable. There appears to be a continuum of size, from the
smallest picornavirus to the large Mimivirus and Megavirus, larger than the smallest free-growing bacterium [10].

Emile Roux and Louis Pasteur also developed a vaccine against the rabies virus, a filterable agent. This vaccine was tested on a young boy, Joseph Meister, who was bitten by a rabid dog and was certain to die. He was given many shots of the vaccine into his belly and he survived the attack. There was much controversy at the time, since some considered the treatment unethical because the vaccine had not been properly tested [11]. However, the argument was that without this, the boy would have died. Although Pasteur had carried out vaccine experiments on dogs, the claim was made that this was not the vaccine used in the case of the boy. However, its success led to its adaption as a mode of treatment until very recently and enhanced Pasteur’s reputation.

Yersin, a researcher at the Pasteur Institute, went on to discover the bacterium involved in the plague (Black Death) in 1894, initially called Pasteurella pestis but renamed Yersinia pestis in 1967. He isolated the bacterium from rats and showed that it was identical to the bacterium associated with the bubonic plague in humans. Thus the foundations of bacteriology and immunology were laid without seeing the organisms involved, and without differentiating virus from bacterium.

### 1.7 Plant Viruses

Pioneering work in microbiology was also carried out on the tobacco mosaic virus (TMV). This was a “germ” that caused areas of variegation (mosaic) and wilting of tobacco plants, a crop of economic importance. Many plant viruses, all species-specific, cause similar symptoms, the most famous of them the virus that causes the variegation of tulips. The pattern that developed as the result of the infection had great economic importance. In fact, it was the rarity of such tulips that led to the market concept of futures, and to the exchange of large sums of money in the seventeenth century in Holland (1634–1637), during a period known as tulipmania. Such tulips did not breed true, and the pattern died out quite suddenly. This caused a financial crash, and may be thought of as the first stock market crash or bursting of a financial bubble [12] (Fig. 1.11).

None of this related historical experience was understood at the time that TMV became a topic of scientific, and perhaps commercial, concern in the 1890s. Adolf Mayer (1843–1942), director of the Agricultural Experimental Station in Wageningen, Holland, first discovered that TMV was an infectious agent. He is credited with being the first person to extract juice (sap) from infected plants and used it as an inoculum to infect other plants. He tried to culture the organism but failed to grow it on media and it was not visible in the then crude microscopes. He concluded wrongly that the agent was a bacterium that lost activity upon filtration [13].

Dmitri Ivanovski (1864–1920), a Russian scientist, also reported that the infectious agent for tobacco mosaic disease was filterable (1892). However, like Mayer, he concluded that there must be something wrong with the filter, or that the agent could not be grown in vitro, or was some kind of spore-forming organism.
It was Martinus Beijerink (1851–1931) who made the seminal discovery that the agent was a “contagious living fluid.” Since the filters should remove known bacteria, he used the term “virus” (the Latin word for “poison”) to describe such fluids. TMV went on to be a very important tool in the study of virology and molecular biology. [13] Beijerink went on to make many major contributions to microbiology, including the discovery of nitrogen fixation, the process by which nitrogen in converted by bacterium (rhizobium) into ammonia, a form that plants can metabolize—a natural organic fertilizer! (Fig. 1.12).
1.8 Human and Animal Viruses

The first animal virus (filterable) shown to be infectious was foot and mouth disease virus (FMDV). This virus has great economic importance, particularly in the dairy industry of western Europe today. While investigating outbreaks of foot and mouth disease virus in cattle in 1897–1898, Friedrich Loeffler (1852–1915), (who had previously worked with Koch and helped form the “Koch Postulates”), together with Paul Frosch (1860–1928), described an agent from the pockmarks of diseased animals that could not be cultivated on the media devised for growing bacteria. This agent was filterable, and could be shown, by dilution, to be infectious. By sequential inoculation from animal to animal and serial dilution, these researchers proved that this was not a toxin but an infectious agent. Thus FMDV resembled TMV in character. By the turn of the twentieth century, it had been established that viruses were infectious agents associated with many diseases [14].

As we will see in a later chapter, among the first viral diseases to be characterized and prevented was yellow fever, identified as an infectious disease in 1900, although it took many years before the actual virus was isolated. This would have to wait until viruses could be grown in cell culture.

1.9 Viruses and Cancer

In parallel with the work described above, major advances were being made in research linking viruses to cancer. The relationship between cancer and viruses was first discovered in an avian species (domestic poultry). Unfortunately, the relevance of disease in non-humans, particularly in non-mammals, to humans was not realized until 50 or 60 years later. We now know, through examples such as “avian flu” or “swine flu,” that other animals are stricken with the same vectors as man, can transmit these vectors to man, or act as a reservoir for these diseases. The first reported case of a possible virus related to cancer was the observation in 1908 by two Danish veterinary researchers, Vilhelm Ellerman and Oluf Bang, who found that leukemia of chickens could be transmitted by a cell-free extract. Peyton Rous (1879–1970) at the Rockefeller Institute in New York reported a few years later that not only leukemia but also solid tumors (sarcomas) were transmissible [15].

Peyton Rous came from a poor Texan family, settled in Baltimore, and received a BA from John Hopkins University in 1900. Due to an accident that affected his health, he worked at a Texas ranch. Returning to medical school, he received his MD degree in 1905 and then worked in the Department of Pathology at the University of Michigan, and later in Dresden, Germany. Subsequently he received a grant to do research at the Rockefeller Institute in New York. The director of the Institute, Simon Flexner, who headed the cancer laboratories, asked him to take his place since he, Flexner, wished to devote his time to poliovirus research, and Peyton Rous succeeded him. While working with spontaneous tumors of chickens, he demonstrated in 1910 that a cell-free extract made from the tumor was
transmittable, and that different types of tumors arose from different cell-free extracts of different tumors. Thus the work of two groups (Ellerman/Bang and Rous) laid the groundwork for an analysis of the relationship between tumors and viruses.

Rous received the Nobel Prize in Medicine in 1966, many years after his discoveries. Not only did he discover tumor viruses (the Rous sarcoma virus [RSV], the first prototype retrovirus discovered), his research also contributed greatly to methods of storing blood, crucial during World War I. He gave up attempting to isolate mammalian tumor viruses a few years after identifying RSV. It took another 50 years or so before tumor-causing viruses were discovered in mammals.

1.10 Bacteriophage

The fact that all organisms can harbor viruses was confirmed by the discovery of bacterial viruses, known as bacteriophage. These were first discovered by Frederick Twort (1877–1950) [16] in England and by Felix d’Herelle (1873–1949), a Canadian working in France. In 1910 d’Herelle, working in Mexico, was alerted to the presence of dysentery in locusts [17]. He cultured the organism from the insects, and noted during the growth of the bacteria that there were areas of clearing in the culture in Petri dishes; however, he did not realize the importance of the discovery until World War I, when he was working with *shigella*, a bacterium that also caused dysentery in humans. D’Herelle realized then that the areas of clearing were plaques caused by a filterable agent, namely a virus-infecting bacteria [18]. He also noted that the culture of bacteria cleared during this infection so that no bacteria were left (or so it appeared). D’Herelle had hoped that such bacteriophage could be used in therapy against bacterial diseases [19]. This was an active area of research in the 1930s–1980s in Eastern Europe and Russia and today is an area of renewed research. The same idea was presented in Sinclair Lewis’s novel *Arrowsmith*, in which a physician experiments with bacteriophage to cure common diseases [20].

While working with cultures of *Staphylococcus aureus* (the bacterium that causes the common boil) in 1915, Frederick Twort noticed that colonies of these bacteria were being destroyed, and, like d’Herelle, he found that clear plaques were formed. He isolated the substance that produced this effect and found that it was transmitted indefinitely to subsequent generations of the bacterium. He then suggested that the substance was a virus. Twort was unable to continue this work, and the importance of bacteriophage was not recognized until the 1950s, when it became a major tool of molecular biologists and revolutionized the whole field of biology. In chapters on bacteriophage (Chaps. 4 and 16), I describe the controversy surrounding the discovery of bacteriophage, its possible use in the treatment of bacterial infections, and the role bacteriophage plays in molecular biology.
1.11 Modern Molecular Biology

An understanding of the molecular organization of viruses began with the crystallization of the tobacco mosaic virus by Wendell Stanley in 1934. Working with highly purified TMV, he was able to identify conditions where virus particles are arranged in a lattice, so that their molecular features can be elucidated by the diffraction of X-ray beams. Wendell Stanley received the Nobel Prize in Chemistry in 1946 for his seminal work. Even at that time it was still believed that proteins were the genetic material, although the virus did contain some nucleic acids. It was not until further work with bacteriophage, and simultaneously with bacteria, that it could be shown that the nucleic acid contained the genetic information. These were experiments involving DNA transformation in bacillus [21], and in later experiments with bacteriophage, it could be shown, using radio-labeled material, that the DNA of the bacteriophage entered the bacterial cells and replicated and produced progeny phage and not the protein [22] that remained attached to the outside of the cell (Fig. 1.13).

The advent of modern molecular biology came about with the analysis of bacteriophage growth and replication. Using bacteriophages such as T2 and lambda, it was possible to dissect each stage of virus replication, the role of nucleic acid in the process, quantify the appearance of and the mechanism of mutation, and develop a model from phage for tumor development, based on the fundamental observation that some phage genetic material was integrated into the bacterial genome. These early studies with bacteriophage, and the parallel studies

![Fig. 1.13 Diagram of the Hershey Chase experiment](http://de.wikipedia.org/wiki/Datei:Hersheychaseexperiment.gif) | Date = 26 April 2011 | Author = Thomasione | Permission = {{GFDL-user-w|de|/wikipedia.org|}}
with cell culture and the growth of viruses, allowed for the quantization of virus growth. The number of scientists involved is too many to list, but all the principal players constituted the phage group, headed by Max Delbruck, that met at the Cold Spring Harbor Laboratories in New York. They were later joined by James Watson and Francis Crick of DNA double helix fame.

### 1.12 Viral Studies in the 1950s

The great virus scourge of the 1940s and 1950s was poliovirus (see Chaps. 3 and 12 on cell culture and poliovirus). A campaign in the United States, spearheaded by the March of Dimes, was determined to find a vaccine against polio. Based on the earlier work of John Franklin Enders and others, in 1953 Jonas Salk announced the achievement of a polio vaccine [23]. Salk became not only a celebrated scientist, but also a hero. His vaccine was based on a “dead” virus, which was followed two years later by a rival vaccine, the Sabin vaccine, based on a series of attenuated (weakened) viruses [24]. Both vaccines have their pros and cons, and both are still used today, although in the U.S. the Salk vaccine is considered safer since it does not involve viruses that can reproduce. However, the introduction of these vaccines was not without risk since the first batch of commercially produced Salk vaccine contained some improperly inactivated virus particles.

As a result of these vaccines, polio has almost been wiped out, although hopes to completely eradicate the virus have recently met with setbacks. Those carrying out the eradication campaign have been murdered in Pakistan, and in other countries the anti-polio inoculation campaign has been viewed as a political or cultural threat. Another oral vaccine was developed by Hilary Koprowski and was used in Africa, but not in the U.S. [25]. This later led to a controversy as to whether the AIDS virus (HIV) arose from the chimpanzee cells used to grow the polioviruses, and was thus introduced into the human population.

A major mystery surrounding viruses was (and to some extent still is) their involvement in cancer. Rous’s sarcoma virus, and later other viruses associated with cancer in mice, were shown to be RNA viruses (at one time it was thought that most viruses were DNA viruses). In culture, these viruses were inhibited by Actinomycin D, a drug known to inhibit DNA-dependent RNA polymerase, the enzyme involved in mRNA synthesis from DNA, suggesting that these RNA viruses had a DNA stage. Although this was difficult to believe, eventually two groups in 1970 independently discovered an enzyme in infected cells (but not in uninfected cells) that copied RNA into DNA by a complicated mechanism. This was “reverse transcriptase,” and the name “retroviruses” was given to this class of virus. The two labs involved were those of David Baltimore and Howard Temin, classmates from Swarthmore College in Pennsylvania [26, 27]. Each made independently parallel discoveries and shared the Nobel Prize for their work. The discovery of this enzyme opened the way to studies of cancer viruses, some of
which could be linked to human leukemia (HTLV-1) and later on to HIV, the virus causing AIDS.

The 1970s to the present was an era of major discoveries and technological break-throughs, and most of the new advances were due to the emergence of molecular biology. The technological advancement is DNA sequencing that allowed the sequencing of the DNA and RNA of thousands of viruses. DNA sequencing can now be done very quickly and inexpensively. The development of the “polymerase chain reaction” (PCR) revolutionized the identification of specific genes, the measurement of gene expression, and virus identification, because nucleic acids that were previously in short supply can be amplified and studied carefully. Specific antibodies could be made in cultured cells (hybridomas) using cell culture techniques. New emerging viruses were discovered and linked to specific diseases or plagues (such as Ebola, HIV, Nypah, Hantan, all of which are known as “emerging viruses”).

All of these topics will be covered in more detail in their respective chapters. What is the situation at the time of this writing?

**Smallpox.** This is the only viral disease that has been wiped out worldwide. However, there is still the fear that this virus will be used in biological warfare, or by terrorists; therefore, stockpiles of the vaccine are necessary, and new methods of producing the vaccine in culture are necessary for stockpiling. Although vaccination has stopped, there are now calls for it to be reintroduced.

**Measles.** An effective vaccine has been available since 1963; this disease could be eliminated with a worldwide effort. However, false rumors based on poor science relating the vaccine to autism inhibited the vaccination program for some time. The linkage to autism was proven to be false.

**Influenza.** Strain-specific vaccines are available, but new variant strains emerge periodically and there are significant animal reservoirs worldwide (aquatic birds). Our battle with the flu, including pandemic flu, will be an ongoing one for the foreseeable future. New vaccines are produced each year based on predictions, and newer technologies, such as cloning of specific viral genes resulting in newer vaccines, are being clinically tested.

**Polio.** Effective vaccines are available. Scientists are optimistic that this will be the second viral disease to be completely wiped out. However, there are cultural problems and resistance to vaccination in certain parts of the world. Efforts are currently focused on replacing the Sabin vaccine with the Salk vaccine in hopes of removing attenuated poliovirus, although the oral Sabin vaccine has a “herd” effect and may thus spread in a population and be more useful in Third World countries. Vaccinating has been held up by civil wars and mistrust of the motives for vaccination.

**HIV.** A massive effort to develop vaccines is under way. There are effective drugs that can be costly and toxic, but they do allow for a normal life span and chronic condition. Worldwide spreading continues via sexual contact and use of recreational drugs. An estimated 250,000,000 people have been infected thus far, with over 30 million deaths. However, with changes in sexual behavior in some
parts of the world, the use of condoms, and the encouragement of male circumcision, this disease is on the decline. With time, anti-retroviral drugs will become more available and cheaper. In the U.S., the disease is now often considered a chronic infection that gives a feeling of false security, that the disease is no longer dangerous of lethal.

**Ebola.** No vaccine currently exists. There is a need to identify the host reservoir, possibly bats. A vaccine is being developed.

**Hepatitis C.** This is now considered the number one challenge in the U.S. and other developed countries. Spread by the use of shared needles among drug users, it takes a long time, perhaps 10–20 years, before the symptoms of the disease are recognized; it can lead to liver cancer.

New viruses are being discovered and entering the human population continuously. As man moves into new environments, or changes social mores, new viruses are likely to exploit these situations and lead to new pandemics.

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