Introduction to Virology

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Preface

The study of viruses, or virology as it is now called, had its origin in 1892 when a Russian botanist, Iwanawsky, showed that sap from a tobacco plant with an infectious disease was still highly infectious after passage through a filter capable of retaining bacterial cells. From such humble beginnings the study of these 'filter-passing agents', or viruses, has developed into a separate science which rivals, if it does not excel, in importance the whole of bacteriology.

The importance of viruses lies not only in the diseases they cause in every type of living organism, but also because of their intimate relationship with the living cell, in which alone they can reproduce. Their study has influenced the whole of biology by greatly increasing our knowledge of the gene, genetics, and molecular structure; there is also the possible connexion of viruses with human cancer, in view of the occurrence of many viral cancers in other animals.

The book attempts to give a comprehensive but necessarily superficial survey of the subject as a whole and should help senior undergraduates and postgraduate students who wish to gain some knowledge of virology. Further information is available from the extensive bibliography.

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Kenneth M. Smith
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Chapter 1

Introduction

Viruses are no modern phenomenon, their existence can be traced back for thousands of years. Unmistakeable signs of poliomyelitis have been observed in Egyptian mummies and smallpox, a typical virus disease, was described by the Chinese in the tenth century before Christ. Virologists in the United States working with a virus which appeared unexpectedly in a culture of cat cells, have discovered some curious properties of the virus which have led them to trace it back 10 million years to its probable evolutionary origin in a baboon.

Yellow fever, caused by one of the arboviruses (arthropod-borne) has been known for centuries as a scourge of ships in the African trade, and Bedson suggests that it was probably responsible for the legends of the cursed ships, the *Ancient Mariner* and the *Flying Dutchman*.

Owing to the confusion of influenza with other similar fevers it is not possible to trace it very far back in human history. It is, however, known to have been present in Europe during the sixteenth century.

The earliest record of a plant virus disease goes back to about the middle of the sixteenth century, though, of course, no conception of such a thing as a virus existed at that time. This refers to a variegation in the flower colour of tulips now called a 'colour break', and is caused by an aphid-transmitted virus. Some of these colour breaks are extremely attractive, and in the early days of the tulip in Holland such tulips fetched absurdly high prices. This led to much financial speculation, a period known as 'tulipomania'.

The development of the microscope and the subsequent discovery of minute organisms such as bacteria led to the further discovery that some of these bacteria could cause disease, and, moreover, that these diseases were infectious. In consequence it was concluded that all infectious diseases were caused by bacteria or 'germs'. It was therefore surprising to Koch and Pasteur that in certain infectious diseases of man and animals no bacteria or 'germs' could be found.

As an explanation for the absence of visible germs, Pasteur, in his study of rabies, suggested the possible existence of minute organisms beyond the resolving power of the microscope of his time. The idea that there might exist a pathogenic agent of quite a different nature did not occur to him. It is worth commenting here that Pasteur's suggestion that there might exist a group of minute organisms beyond the resolving power of the optical microscope was after all correct, though not of course as a cause of rabies. There has been discovered comparatively recently a group of ultra-microscopic organisms, the mycoplasmata, which cause disease, and many plant diseases hitherto attributed to virus infection are in fact caused by these organisms.

The first scientific demonstration of the existence of 'filtrable viruses', as they came to be called, was made in 1892 by Iwanowsky, a Russian botanist, who was working with a disease of the tobacco plant known as 'mosaic'. This disease had been shown previously by Adolph Mayer, about 1886, to be infectious to healthy tobacco plants if infected sap was rubbed on the leaves. Iwanowsky expressed the sap from a mosaic-infected tobacco plant and passed it under pressure through a Pasteur-Chamberland filter candle. The clear fluid thus obtained was sterile, free of all bacteria. Much to his surprise Iwanowsky found that this apparently sterile fluid was just as infectious, when rubbed on the leaves of healthy tobacco plants as was crude unfiltered sap. Iwanowsky seemed singularly unimpressed by his discovery and merely concluded that he was dealing with bacteria of unusually small size; a view, incidentally, held by some biologists till the electron microscope was developed and viruses became visible for the first time.

Iwanowsky little realized that he had opened up an entirely new field of research, one that, through the intimate relationship of the virus with the living cell, was to revolutionize the whole of biology and to throw light on genetics and the structure of the gene.

A quotation here is apposite. It comes from the Penrose Memorial Lecture delivered in 1957 by W.M. Stanley who was the first to isolate a virus, that of tobacco mosaic. He said: 'The study of virus nucleic acid is vastly more important than any of the problems associated with the structure of the atom, for in nucleic acid structure we are dealing with life itself and with a unique approach to bettering the life of mankind on earth.'

Iwanowsky's work was carried a stage further by a Dutch microbiologist, M.W. Beijerinck, who confirmed Iwanowsky's filtration results, but demonstrated in addition that the mosaic virus would diffuse through a thick agar layer, leaving as a residue all discrete particles, aerobic and anaerobic bacteria and their spores. From these results he conceived the idea of a *contagium vivum fluidum*. Although this conception is not very precise in its meaning, it is important as being the first step away from the conventional bacteriological approach to the problem. At about this time two German workers, Loeffler and Frosch, had shown that the foot-and-mouth disease of cattle was also caused by a filter-passing agent. Beijerinck took issue with Loeffler and Frosch for calling their agent 'corpuscular'. In the next chapter it will be seen that the sizes of Beijerinck's 'vivum fluidum' and Loeffler and Frosch's 'corpuscles' are respectively 300 × 15 nm and 22 nm.

Viruses are now known to attack every kind of living organism. The whole animal kingdom (including, of course, man himself) and the whole plant world are susceptible to virus infection.

In addition, viruses have been found in all types of micro-organisms, especially bacteria, but also in algae, fungi and protozoa such as amoebae and paramecium. Recently viruses have also been found in the mycoplasmata; these micro-organisms are themselves ultra-microscopic and, as already mentioned are, or may be, pathogenic.

Here surely is the *infinitum* of the old rhyme about 'bigger fleas have lesser fleas' etc. – unless, of course, the unlikely and probably impossible situation arises of one virus parasitizing another.

1.1 VIRUS CHARACTERISTICS

Viruses can be differentiated from other pathogens by the following properties. Their size puts all plant viruses and most of the animal viruses beyond the resolution of the optical microscope. (There is, however, a group of potentially pathogenic organisms previously mentioned, the mycoplasmata, which are also ultra-microscopic.)

Viruses have an extremely close affinity with the living cell, outside which they cannot reproduce. No virus has ever been cultivated in a cell-free medium. Their chemical constitution is simple and in the very small viruses consists of a single molecule of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) contained inside a protein coat made up of numerous identical copies of a single protein. The 'viroids' appear to consists of a strand of naked nucleic acid, the virus genome, without a protein coat.

Many of the small viruses behave like chemicals and can be crystallized.

Finally, there is in many cases a close biological relationship between the virus and its arthropod or other type of *vector* on which the virus depends on its transport from host to host.

1.2 SOME VIRUS DEFINITIONS

There have been many attempts made to arrive at a satisfactory definition of a virus, and some of these are given here.

In their definition, Lwoff and Tournier (1966) list the following features which are absent in other agents such as bacteria, agents of psittacosis, protozoa etc., but are present in all viruses. (a) Virions possess only one type of nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA); other agents possess both types. (b) Virions are reproduced from their sole nucleic acid, whereas other agents are reproduced from the integrated sum of their constituents. (c) Viruses are unable to grow or to undergo binary fission. (d) In viruses the genetic information for the synthesis of the Lipman system, the system, responsible for the production of energy with high potential, is absent. (e) Viruses make use of the ribosomes of their host cells; this is defined as absolute parasitism.

Luria and Darnell (1968) define viruses as entities whose genome is an element of nucleic acid, either DNA or RNA, which reproduces inside living cells and uses their synthetic machinery to direct the synthesis of specialized particles, the virions, which contain the viral genome and transfer it to other cells.

Bawden (1964) suggests a shorter definition as being more suitable for the plant viruses: 'They are sub-microscopic infective entities that multiply only intracellularly and are potentially pathogenic.'

On the other hand Hahon (1964) considers that definitions based on size, pathogenecity and obligate parasitism are inadequate. Viruses are now envisaged as transmitters or vehicles of information-bearing genetic material or, stated in more general terms, as 'bits of infectious heredity in search of a chromosome.'

It may be helpful here to emphasize those virus characteristics which are common to all the definitions. Viruses contain only one type of nucleic acid, DNA or RNA, never both, they can only replicate inside a living cell and they are obligate parasites.

Chapter 2

Some representative viruses – size, morphology, ultrastructure and replication

2.1 SIZE

As a first step it will be advisable to define the various constituent parts of the virion. As already stated in the Introduction, all viruses contain nucleic acid, either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), but never both. The nucleic acid is contained within a coat made up of large numbers of similar protein subunits known as *capsomeres*. The whole package is called a nucleocapsid. The capsomeres themselves are composed of smaller particles known as *structural units*. In the case of the isometric viruses, icosahedral (twenty-sided) or near spherical, the capsomeres are arranged in a box-like form, called a *capsid*.

There is much variation in the sizes of virus particles; the parvoviruses, so-called, measure only 18–22 nm (nanometers) in diameter. Not counting the 'viroids' which appear to consist only of a naked strand of nucleic acid without a protein coat (see Chapter 8), probably one of the smallest viruses is that which attacks the tobacco plant and is concerned with the disease known as 'tobacco necrosis'. This virus measures only 17 nm in diameter but is unable to replicate by itself and needs the presence of another similar but complete virus. This is called 'satellitism' (see Chapter 8).

Other very small viruses are those causing the denso-nucleosis of the larva of the moth *Galleria mellonella* (20 ± 1.5 nm), the foot-and-mouth disease of cattle (22 nm) and the bushy stunt disease of tomatoes (30 nm). These are isometric viruses, near spherical or icosahedral in shape, and so it is possible to give one measurement of size, the diameter. In measuring the anisometric viruses, however, it is necessary to give the length and thickness of the particle.

The largest viruses are the pox viruses which attack the higher animals and also insects; these latter are known as 'entomopoxviruses'. They measure about 400 × 250 nm. The large animal-pox viruses or 'elementary bodies' were the first viruses to be actually seen and so to substantiate the suggested 'corpuscles' of Loeffler and Frosch.

They were photographed by the late J.E. Barnard on his ultra-violet light microscope; they appeared, however, only as minute particles and no details of structure were visible. In 1931, before the introduction of the electron microscope and thus before the visualization of viruses was possible, a method of measuring the size of some viruses was developed. W.J. Elford introduced a new type of collodion membrane filter of great uniformity and reproducibility. The pore size of these 'Gradocol membranes' could be accurately measured and a relationship between the size of the virus particle and the pore size of the membrane which just retained it was deduced. By this filtration process the sizes of a number of animal and plant viruses was calculated and these sizes have since been confirmed by the electron microscope and other modern techniques. For example, the size of tomato bushy stunt virus, as measured by Elford's method, was found to agree closely with the measurement obtained later by means of the electron microscope. However, difficulties arose when attempts were made to measure, by means of these membranes, viruses which were later found to consist of long rods, such as potato virus X (PVX) or tobacco mosaic virus (TMV). How recently acquired is all the present-day information on the sizes, shape and structure of viruses can be gauged by a remark of the late T.M. Rivers who stated in 1932, with a good deal of justification that 'the size of no virus was accurately known.'

2.2 MORPHOLOGY

There is as great a variation in the shape of virus particles as there is in their size.

The first indication of the shape of a plant virus was given by two American workers, Takahashi and Rawlins in 1933. They observed that the sap of a tobacco plant, infected with the mosaic disease, showed the phenomenon of double refraction, or 'anisotropy of flow' when made to flow and viewed by polarized light. This indicated that the sap contained rod-like particles which orientated themselves rather like logs would do in a flowing stream; as will be realized later the virus of tobacco mosaic has been studied more intensively than any other virus with the possible exception of the bacteriophages (or phages as they are usually called).

The very small viruses appear to be near-spherical or octahedral in outline. In actual fact, however, most of these are icosahedral, possessing twenty sides.

The first demonstration of an icosahedron was made by Williams and Smith (1958). They used one of the large isometric viruses, the *Tipula* irridescent virus (TIV), one of a group with peculiar optical properties. TIV attacks the larva of the crane-fly, *Tipula paludosa* and many other insect species. The virus measures about 130 nm in diameter and its shape was

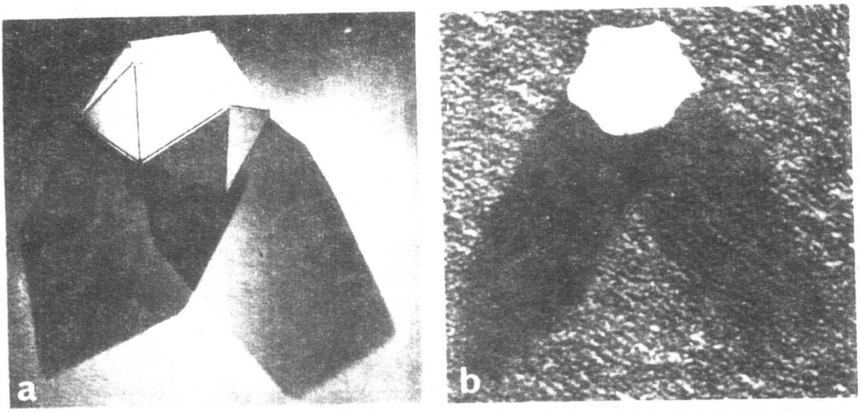

Fig. 2.1 (a) A model of an icosahedron illuminated by two light sources and orientated so that an apex of the hexagonal outline points directly to each source. This throws two shadows: one is four-sided and pointed, and the other is five-sided with a blunt end. (b) A particle of the *Tipula* iridescent virus freeze-dried and shadowed in the same way as Fig. 2.1 (a): the similarity between the shadows thrown is evident. (× 124 000.) (Courtesy of R.C. Williams and K.M. Smith, 1958.)

demonstrated by electron microscopy using a method known as 'double-shadowing' which was carried out as follows. A model of an icosahedron was made and shadowed by two light sources separated 60° in azimuth and orientated so that an apex of the hexagonal contour points directly to each light source. This throws two shadows, one is four-sided and pointed and the other is five-sided with a blunt end. (Fig. 2.1(a)). A particle of TIV, freeze-dried and shadowed with metal in the same way is shown in Fig. 2.1(b). The similarity between the shadows thrown is evident. This indicates with fair certainty that the TIV particle is an icosahedron.

The viruses causing the cytoplasmic polyhedroses (CPVs) of insects all appear to be icosahedra but with the addition of a number of projections. In the case of the CPV affecting the larva of *Danaus plexippus*, the Monarch butterfly, there are twelve projections, each measuring about 17 nm in length. In section, the virions often appear as a six-pointed star. An unusual feature is the possession of a 'tail' extending from a vertex of the hexagon; the length of the 'tail' is almost that of the virus particle itself and is much longer than the regular projections (Arnott, Smith and Fullilove, 1968).

Many viruses affecting both animals and plants are rod-shaped but there is great variation in the type of rod. They have been described as helical, tubular, bacilliform or bullet-shaped; and a number of viruses of the latter type have been grouped together as the 'Rhabdoviruses' (from *rhabdos*, a rod).

Among these may be mentioned the virus causing vesicular stomatitis in horses and cattle in Central and North America. The recently isolated

Fig. 2.2 Virus inclusions in the cytoplasm of a mesophyll cell of *Lantana horrida* infected with a mosaic virus. Note the remarkable helices. (*After* Arnott and Smith, unpublished.)

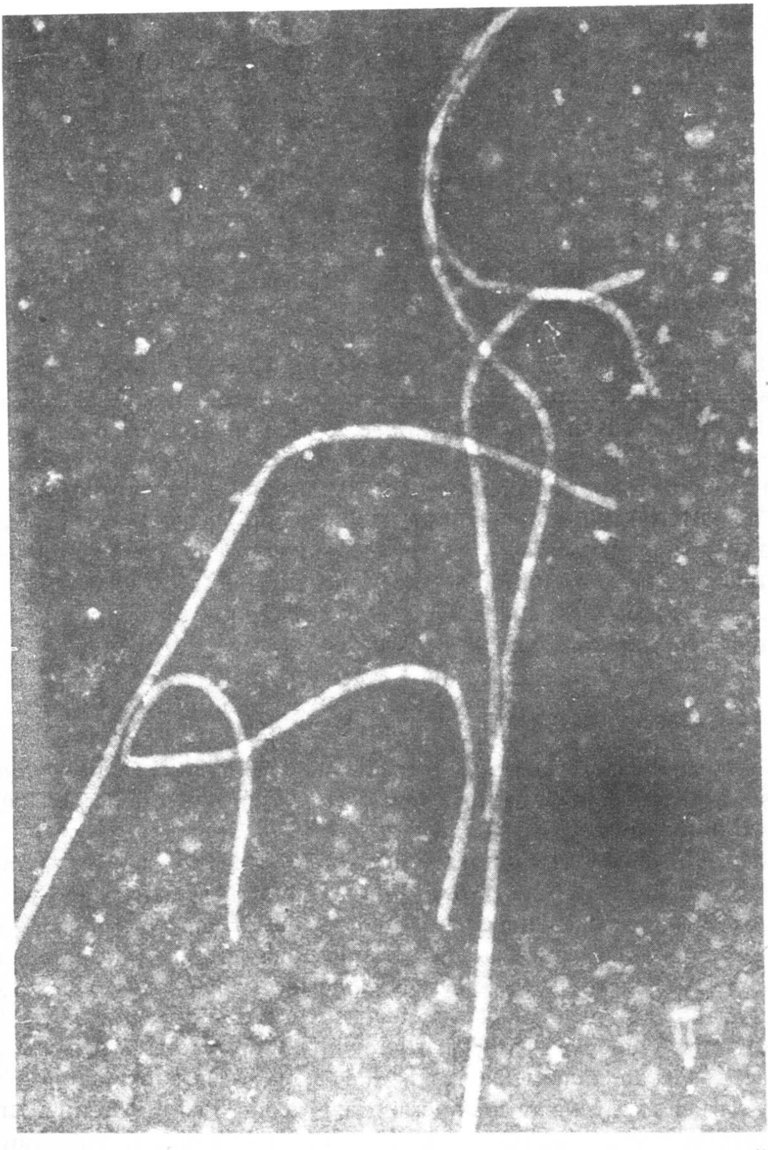

Fig.2.3 The long, thin, thread-like virus which is the cause of sugar beet-yellows. (*Courtesy* of the John Innes Institute.)

Marburg virus, the cause of 'green monkey disease', has many of the characteristics of rhabdoviruses but is of extreme length (Fig. 2.1). The virus of rabies is bullet-shaped with one round and one flat end; this puts it into Hull's (1976) category of tubular viruses.

Examples of very long viruses are those of two plant diseases, *Lantana* mosaic (Fig. 2.2) and sugar beet yellows (Fig. 2.3) and that of the green monkey disease mentioned above.

The large pox viruses affect animals including birds and insects. Those which attack insects ('entomopoxviruses') are morphologically similar to, but biologically different from, the pox viruses which affect the higher animals. Pox viruses are oval or brick-shaped and more details of their appearance and structure are given later in this chapter.

The virus of chronic bee paralysis is ellipsoidal in shape, often with a small protuberance at one end. Projections or protuberances occur in several viruses, especially in those attacking insects. In the polyhedral diseases (polyhedroses) there is often a prolongation of one end of the virus rod (nuclear polyhedroses) and, as already stated, there may be as many as twelve projections as well as a 'tail' on the icosahedral virion in the cytoplasmic polyhedrosis (CPV) of the Monarch butterfly (*Danaus plexippus*). Projections but no 'tail' occur in the CPV of the silkworm (*Bombyx mori*).

The bacterial viruses (phages) are the most complex and one of the most studied groups of viruses. A T_2 phage consists of an icosahedral 'head' containing DNA, a contractile 'tail' and a number of other structures described in more detail later. Fig. 2.4 is a diagram showing the shape and comparative sizes of a number of viruses.

2.3 ULTRASTRUCTURE

The very small virus which attacks the larva of the moth *Galleria mellonella* causes a disease known as 'densonucleosis' because of the dense changes caused in the cell nuclei.

The following description of the structure of the virion is taken from the work of Kurstak and Côté (1969). It has icosahedral symmetry and the full particle has a capsid composed of closely linked capsomeres measuring 2·0–3·5 nm with a central hole of 1·5 nm diameter. The total number of capsomeres composing the full DNV capsid is calculated to be 42, 30 hexagonal and 12 pentagonal capsomeres (Kurstak and Garzon 1971). Fig. 2.5(e) shows a model of a DNV particle with 42 capsomeres, 12 vertices and 20 faces, each an equilateral triangle. Fig. 2.5 is an electron micrograph of DNV particles showing an equilateral triangle composed of 6 hollow-centre capsomeres with 3 capsomeres on each edge. The long arrow indicates a vertex pentamer corresponding to the structure portrayed in the model.

The DNA particle is thought to contain single-stranded DNA. It has also been suggested that two complementary strands were present separately in different particles of DNV (Barwise and Walker 1970; Kurstak *et al.* 1971; Keller *et al.* 1977).