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Structure and Function of Viruses

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General Preface to the Series

It is no longer possible for one textbook to cover the whole field of Biology and to remain sufficiently up to date. At the same time teachers and students at school, college or university need to keep abreast of recent trends and know where the most significant developments are taking place.

To meet the need for this progressive approach the Institute of Biology has for some years sponsored this series of booklets dealing with subjects specially selected by a panel of editors. The enthusiastic acceptance of the series by teachers and students at school, college and university shows the usefulness of the books in providing a clear and up-to-date coverage of topics, particularly in areas of research and changing views.

Among features of the series are the attention given to methods, the inclusion of a selected list of books for further reading and, wherever possible, suggestions for practical work.

Readers' comments will be welcomed by the author or the Education Officer of the Institute.

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Preface

In the early days, the diseases caused by viruses were considered to be of far greater importance than the causative agents. Moreover, there was little overlap, exchange of ideas and experimental results between medical, animal, plant and bacterial virologists. Today the whole subject of virology has been brought much closer together. There is now a vast list of published material resulting from the extensive application of complex physico-chemical techniques to the study of a wide range of viruses. These studies clearly show the structure, composition and biology of viruses to possess many common features. However, in certain areas of virology, with special reference to structure and function, there are large gaps in our knowledge compared to other biological systems.

The main purpose of this book is to present some basic information concerning the structure and biology of a few selected animal, plant and bacterial viruses. The chapters dealing with the fundamental processes of infection and replication indicate how certain viruses are also being used as experimental systems in some areas of molecular biology.

The author is indebted to many colleagues engaged in research and teaching virology for their helpful and constructive comments during the preparation of this booklet, and not less importantly for supplying many of the illustrations.

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1 Introduction

The first observations that a germ-free fluid extracted from diseased plants could be used to transmit the same disease to healthy leaves were made in 1892 by the Russian botanist D. Iwanowski. In 1898, the Dutchman Martinus Beijerink made use of the Latin word 'virus' (poison) to describe the disease-forming properties of filtered plant fluids or saps. Two other workers, F. Loeffler and P. Frosch, experimented with material from animals infected with foot and mouth disease, confirming that a filterable agent smaller than bacteria was capable of transmitting the disease in cattle. Moreover, they found that very small volumes or droplets of the fluid were required to infect a healthy host.

During the period 1915 to 1917 a series of important experiments were reported independently by F. W. Twort and F. D'Herelle. They observed that cultures of bacteria underwent a 'glassy transformation' which was attributed to a filterable virus. This agent, described as a 'bacteriophage' (bacteria-eater), caused a considerable amount of controversy among bacteriologists and medical practitioners about the potential use of bacteriophages as a method for treating bacterial infection. These differences of opinion stimulated much interest in many bacteriological laboratories concerned with the behaviour of bacteriophages. Although the general idea of using the bacterial virus as a therapeutic system failed, several important and fundamental facts were established. First, the bacteriophage material interacted with the host bacterium in a specific manner. Second, the agent entered the bacterial cell and was capable of multiplication, and third, the bacterial cell ultimately lysed, releasing large numbers of the bacteriophages.

The bacteriophage system offered considerable experimental and practical advantages over the investigations requiring the use of animals and plants for virus studies. These advantages were quickly exploited by several workers who confirmed D'Herelle's concept that the virus multiplied in the bacterial cell. In 1929 F. M. Burnet showed that a single bacterial cell infected with one phage particle produced about 20 to 100 progeny approximately 20 minutes following initial infection. The adsorption of the bacteriophage as an irreversible mechanism was demonstrated later by A. P. Krueger (1930), and M. Schlesinger (1931).

In 1922 F. O. MacCallum and E. H. Oppenheimer described the first successful attempts to purify a poxvirus. About ten years later W. M. Stanley developed techniques which were to provide samples of highly purified tobacco mosaic virus (TMV). This work was followed by the important observations by F. C. Bawden and N. W. Pirie in 1937,

demonstrating that TMV was composed of ribonucleoprotein. The type and approximate location of the nucleic acids found in several viruses were determined by Markham between 1950 and 1953.

In spite of the high performance and advances made in the design and construction of the compound light microscope, it was not possible to resolve any specific 'particles' which could be identified as viruses with the aid of conventional microscopy. However, the large poxviruses were detectable under favourable conditions close to the limit of resolution. The direct visualization of viruses had to await the development of the more powerful electron microscope which appeared in an experimental form between 1932 and 1936. It is interesting to note that bacteriophages were among the first biological specimens to be photographed in the electron microscope. The rapid development of high resolution electron microscopes coupled with specimen preparation techniques provided a wealth of information about virus structure and physical dimensions.

Within a few years of the early bacteriophage and plant virus studies the search for viruses infecting man, animals, plants and bacteria expanded in many laboratories and research institutes throughout the world. In addition to the importance of diseases associated with viral infection, the use of viruses as an experimental system in the field of molecular biology has made spectacular advances.

Interest in virology is still expanding, notably in new methods for the study of virus infection, composition, assembly and structure. These investigations require close collaboration between virologists, cell biologists, biochemists and electron microscopists. Much of the current research involves the use of complex apparatus and highly specialized techniques, discussion of which is outside the scope of this book. Nevertheless, it is hoped that some of the essential features of viruses and their interactions with host cells included in the following discussions will encourage further interest in this important field of microbiology.

1.1 What is a virus?

According to Andre Lwoff 'a virus is a virus'. Viruses can be considered as the smallest organized infective structures capable of replicating themselves in a living cell system. They are composed of either DNA or RNA containing the necessary genetic information for the replication and assembly of identical progeny within the host cell. In order to protect the genetic material the virus possesses a coat of protein or lipoprotein molecules assembled according to precise geometrical or morphological designs. Viral nucleic acid can, under certain experimental conditions, infect cells on its own, and produce complete viruses possessing protein coats.

Viruses are physically very small with an approximate size range of about 20 nm to 300 nm (Fig. 1–1). They can only be resolved directly in

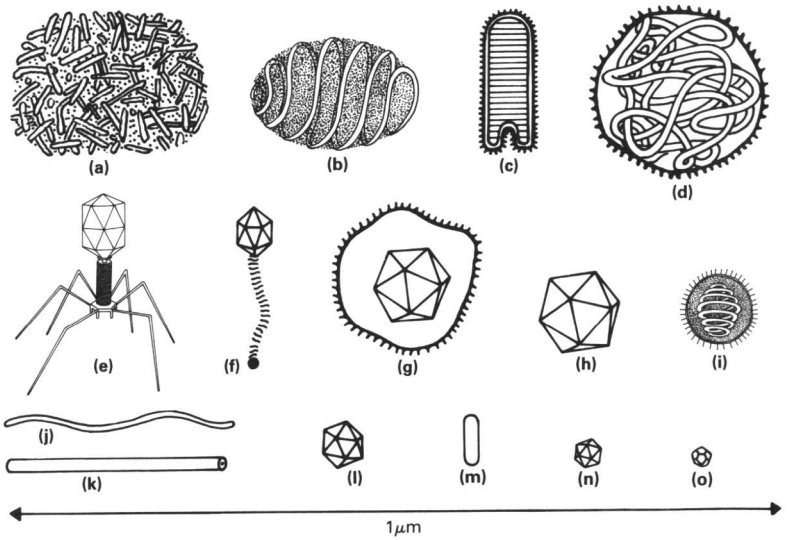

Fig. 1–1 Diagram showing the approximate size and shapes of different viruses. (**a**) poxvirus (vaccinia), (**b**) poxvirus (Orf), (**c**) rhabdovirus, (**d**) para-influenza virus (mumps), (**e**) T-even bacteriophage, (**f**) bacteriophage, (**g**) herpes virus, (**h**) adenovirus, (**i**) influenza virus, (**j**) potato virus X, (**k**) tobacco mosaic virus, (**l**) polyoma/papilloma virus, (**m**) alfalfa mosaic virus, (**n**) polio virus, (**o**) ΦX174 bacteriophage. (Revised from HORNE, R. W. (1963). *Scientific American*, **208**, 48–56.)

the electron microscope at relatively high instrumental magnifications. In order to resolve and measure the size of most viruses and their components the electron microscope image is normally recorded at about ×20 000 to ×40 000. The plate or film can be subsequently enlarged further by normal photographic techniques.

In many cases the virus infects the host cell by direct interaction between the particle and the cell surface, but in other cases viruses are transmitted through an intermediate host. Whether viruses are living, or complex assemblies of macromolecules, is a matter of opinion. The events occurring within a living cell following virus infection form part of a biological process of biochemical synthesis, control and replication. When assembly of virus progeny within the cell is complete, the cell dies and releases a defined packaged structure which remains in a static state until it is able to contact and infect a new host. However, it is possible for virus infection to occur in certain situations where total destruction of the cell does not take place; this will be returned to later. Thus it can be argued that viruses form a borderline between a living and non-living state in biology.

1.2 Virus structure and composition

Before describing the physical virus particle, some general remarks should be made at this point about their structural design. F. H. C. Crick and J. D. Watson made an important comment about virus organization following evidence provided from various physical and chemical studies. They proposed that because the physical dimensions of viruses were very small, they could only contain a limited amount of nucleic acid. The number of viral proteins capable of being coded from the infective nucleic acid was therefore limited, and some form of regular or symmetrical packing of the assembled protein molecules was required to construct the viral shell and at the same time provide maximum efficiency in order to enclose space. Thus, the basic requirement for viruses to appear as regular or symmetrical structures is a biological one.

1.3 Symmetry in virus particles

Much of the evidence for symmetry and regular forms observed in virus particles has come from electron microscopy, X-ray diffraction of virus crystals and physico-chemical studies. For descriptive purposes viruses are considered under three main symmetrical groups: icosahedral, helical, and combinations of symmetrical patterns or complex forms.

1.4 Terminology

Although viruses are comparatively simple structures there is a well-established terminology in current use to describe their various structural

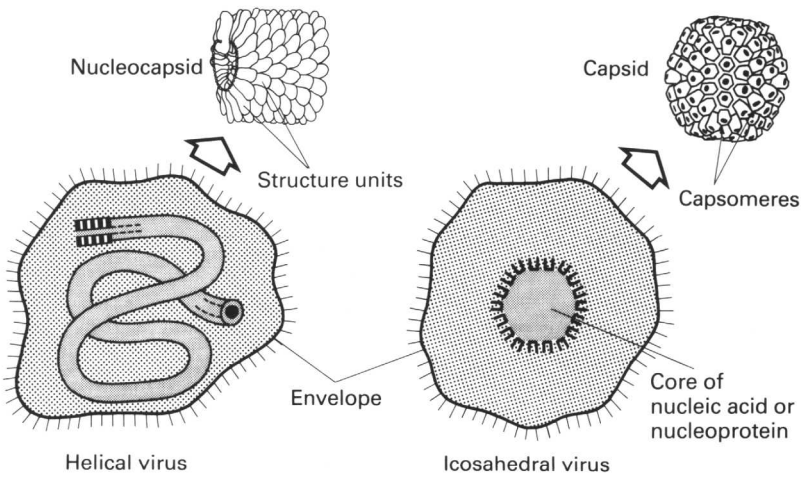

Fig. 1–2 Diagram illustrating the terminology used to describe viruses with helical symmetry (left) and icosahedral symmetry (right).

features and components. The terminology covering the basic features of the icosahedral and helical viruses is illustrated in Fig. 1–2. A complete and infective virus is called a *virion*. In the case of the icosahedral viruses the protein shell is the *capsid*. The capsid is composed of *morphological units* or *capsomeres*. When seen in the electron microscope at a resolution of about 2.5 to 3.0 nm the capsomeres are often in the form of angular prisms, composed of clustered protein molecules or structure units. The structure unit and chemical unit are considered to be the same (Fig. 1–3 a and b). The central part of the capsid containing the nucleic acid genome

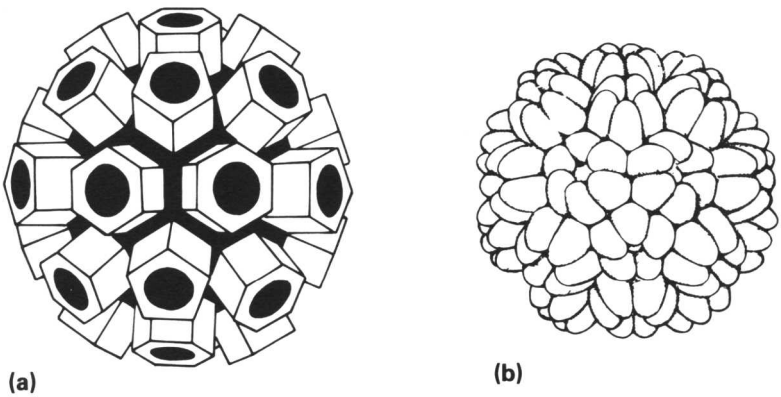

Fig. 1–3 (**a**) Diagram to illustrate the assembly of a plant virus capsid composed of 32 capsomeres or morphological units (30 hexagonal prisms and 12 pentagonal prisms) assembled to form an icosahedral shell. The 32 morphological units are seen in the electron microscope corresponding to a resolution of about 2.5 to 3 nm. (**b**) X-ray diffraction data at a resolution of about 0.5 to 1.0 nm shows 180 structure or chemical units clustered in groups of 6 and 5 to form the 32 morphological units shown in (**a**).
(Reproduced by kind permission of Dr D. L. D. Caspar.)

or nucleoprotein is the *core* or core region. It will be shown later that several groups of viruses possess an additional external *envelope* or membrane derived from the host cell plasmalemma or highly modified plasmalemma.

2 Icosahedral Viruses

As a result of images seen in the electron microscope and analyses of X-ray diffraction diagrams, the 'spherical' viruses belong to a group of structural designs where the capsid is assembled from the structure units or capsomeres to form a series of icosahedra. The regular icosahedron (Fig. 2–1), belongs to the group of five Platonic solids. There are 20 facets as regular triangles, 12 corners or apexes and 30 edges. When viewed along one of the apexes the icosahedron can be rotated about a five-fold symmetry axis. The same body viewed at the centre of one of the regular

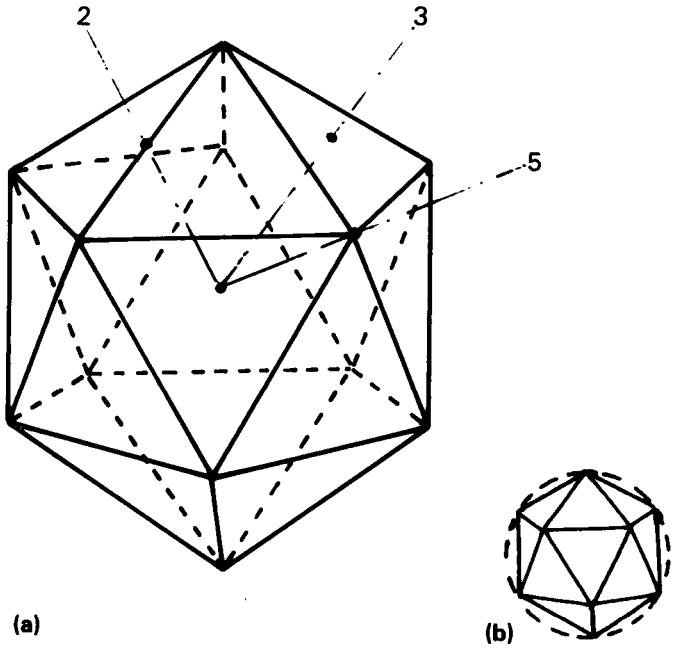

Fig. 2–1 (a) Diagram of a regular icosahedron showing the 2-fold, 3-fold and 5-fold rotational symmetry axes. (b) An icosahedron inscribed within a sphere. The same 5.3.2 symmetry axes shown in (a), can be projected from the centre of a sphere to regular positions at the surface.

triangular facets can be rotated about a three-fold symmetry axis, and also about a two-fold symmetry axis in a central position vertical to the edges. The rotational axes of the icosahedron are referred to as 5.3.2 symmetry.

As with the four other Platonic bodies (cube, tetrahedron, octahedron, dodecahedron), the icosahedron can be inscribed within a sphere. The points of five-fold symmetry or apexes can be positioned to make contact within a sphere as illustrated in Fig. 2–1b. It follows that not only does a body with icosahedral symmetry of roughly hexagonal outline possess 5.3.2 symmetry, but a spherical-shaped object can have the same symmetry axis arrangement distributed as its surface. In addition, it is possible to build an icosahedral body or sphere by placing 12 pentagons at the apexes (five-fold symmetry axes) and *n* number of hexagons on the faces (three-fold symmetry) and edges (two-fold symmetry) (Fig. 1–3). This is also illustrated in the model shown in Fig. 2–2 composed of 12 pentagonal prisms and 150 hexagonal prisms.

2.1 Morphological units and structure units

Proteins, lipoproteins and nucleic acids account for the structural and chemical composition of viruses. Evidence from the X-ray diffraction

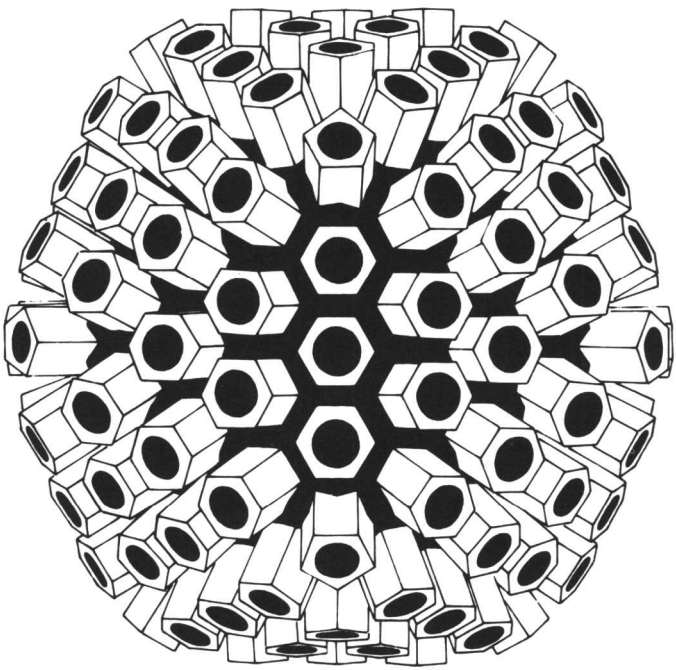

Fig. 2–2 Drawing of a herpes virus capsid composed of 162 capsomeres. There are 150 hexagonal prisms at the faces and edges of an icosahedron and 12 pentagonal prisms located at the apexes. The capsid is about 100 nm across and is viewed along one of the 2-fold symmetry axes.

studies of crystals formed from small icosahedral viruses show the presence of regularly arranged components corresponding to the basic structure or chemical units. The electron microscope images of the same viruses at poorer resolution reveal different structural patterns at their surfaces. The structural patterns are clearly seen at a resolution of about 3.5 to 4.0 nm as groups of pentagonal and hexagonal units distributed in positions consistent with 5.3.2 symmetry. In the case of a plant virus capsid in Fig. 1–3 there are 12 pentagonal units and 20 hexagonal units forming a virus particle of about 28.0 nm diameter. The X-ray and biochemical studies, on the other hand, had established the existence of 180 protein units present in the same icosahedral virus capsid. These numerical differences between the 32 morphological units or capsomeres and the 180 structure units are explained by assuming the capsomeres are composed of clustered structure units in groups of five or six as illustrated in Fig. 1–3b.

2.2 Triangulation numbers

The majority of small icosahedral viruses were considered by D. L. D. Caspar and A. Klug to fall into a theoretical series of icosahedra where the number of capsomeres and structure units could be related to a *triangulation number*. Although the idea of triangulation numbers was considered by Euclid as a system of placing units at the corners, edges and faces of a regular triangle, the Caspar and Klug theory relates to the more complex series of icosahedra. It is possible to show the basic relationship between the total number of capsomeres and structure units in a simple series of examples. These are shown in Table 1.

It was mentioned earlier that the pentagonal and hexagonal grouping of the capsomeres is related to icosahedral (5.3.2) symmetry, There will be 12 pentagonal capsomeres (located on the apexes) on any icosahedral capsid and n number of hexagonal capsomeres (located on faces and edges). On the basis of a regular pentagonal and hexagonal distribution the triangulation number can be determined by multiplying the 12 pentagonal capsomeres by 5 (5 clustered structure units) and the number of hexagonal capsomeres by 6 (6 clustered structure units). The products are then added together and divided by 60. The final numbers 1, 3, 4, 7, etc., shown in Table 1 are the triangulation (T) numbers used to indicate the structural patterns associated with certain groups of icosahedral viruses.

The use of a triangulation number may be a more convenient method for referring to a capsid structural group, but it should be stressed that some viruses may be found which could offer alternative arrangements of their icosahedral grouping in terms of structure units and capsomeres. It is theoretically possible, for instance, to have a common structure unit shared between a pentamer and hexamer forming an icosahedral lattice distribution in the capsid.

Table 1 Calculation of triangulation numbers.

Virus	*Diameter* (nanometres nm)	*No. capsomeres*	*Triangulation number*	
ΦX 174 phage	24	12	$12 \times 5 = \frac{60}{60} = 1$	T=1
Turnip yellow mosaic virus	28	32	$12 \times 5 = 60$ $20 \times 6 = \frac{120}{180}$ $\frac{180}{60} = 3$	T=3
Nudaurelia capensis	50	42	$12 \times 5 = 60$ $30 \times 6 = \frac{180}{240}$ $\frac{240}{60} = 4$	T=4
Polyoma/papilloma	58	72	$12 \times 5 = 60$ $60 \times 6 = \frac{360}{420}$ $\frac{420}{60} = 7$	T=7
Reovirus	60	92	$12 \times 5 = 60$ $80 \times 6 = \frac{480}{540}$ $\frac{540}{60} = 9$	T=9
Herpes	100	162	$12 \times 5 = 60$ $150 \times 6 = \frac{900}{960}$ $\frac{960}{60} = 16$	T=16
Adenovirus	75	252	$12 \times 5 = 60$ $240 \times 6 = \frac{1440}{1500}$ $\frac{1500}{60} = 25$	T=25

3 Helical Symmetry

Helical and spiral systems are sometimes described as representing the same basic forms, but there are important differences between the shape, geometry and symmetry of the helix, spiral and helicospiral (Fig. 3–1). An early example of a simple structure with helical or screw symmetry is the Archimedes pump (Fig. 3–2). This device has a long central axis, is circular in cross-section and of constant diameter. The geometry of the system is helical with a constant pitch of the helix and can be rotated about its long axis.

It should be said here that the tobacco mosaic virus rod shown in Fig. 3–3b is a two-dimensional image recorded in the electron microscope, but it is derived from a three-dimensional helix (Fig. 3–3a). For this reason the pitch of the helix will appear as a series of regularly spaced striations along the long axis of the virus rod. In images of the more loosely assembled flexuous filamentous viruses or nucleocapsids, the helix is presented as a zig-zag pattern (Fig. 5–8). The spiral and helicospiral shown diagrammatically in Fig. 3–1 a and c, obviously present a very different geometrical two-dimensional or three-dimensional arrangement when compared to Fig. 3–3a or helical virus rod in Fig. 3–3b.

In some cases it has been possible to orientate these rod-like or filamentous virus particles from highly concentrated suspensions to form para-crystalline arrays. These crystals were studied by J. D. Bernal and his

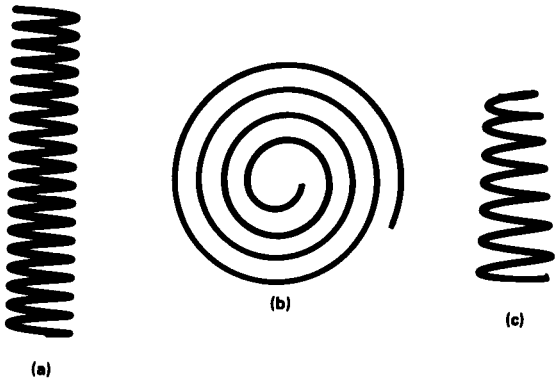

Fig. 3–1 The drawings illustrate the basic differences between the geometry of a helix (**a**), spiral (**b**), and helicospiral (**c**). The spiral (**b**) is 2-dimensional, but structures corresponding to (**a**) and (**b**) are 3-dimensional.

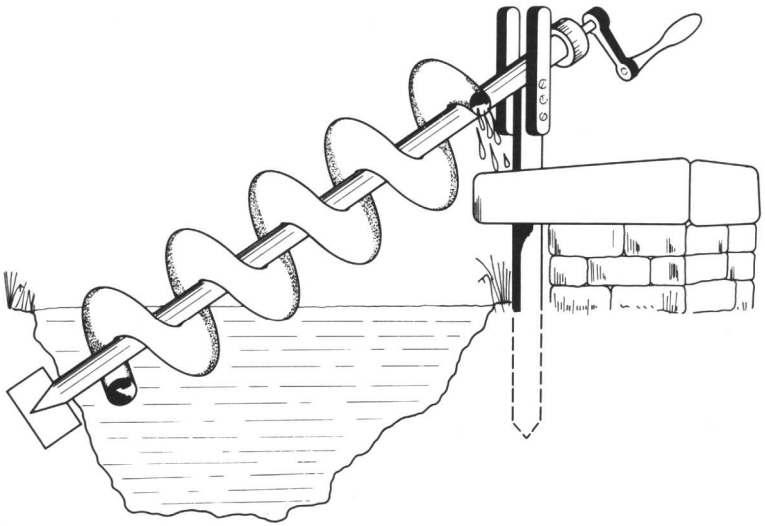

Fig. 3–2 Drawing of an Archimedes screw water pump invented by the Greek mathematician (*c.* 290–212 B.C.) The pitch of the helix and diameter of the device is constant.

colleagues in 1941 with the aid of X-ray diffraction techniques which provided clear evidence for the helical arrangement of the protein and nucleic acid. This pioneer X-ray work paved the way for future detailed analyses to be made on the structure of TMV rods. Long before the specimen preparative techniques allowed the structural features of TMV to be observed directly in the electron microscope, a model of the virus was constructed from X-ray data coupled with biochemical analysis and is illustrated in Fig. 3–3a.

The basic helical arrangement of TMV protein structure units and nucleic acid (nucleocapsid) is to be found in many animal, plant and bacterial viruses. These nucleocapsids are assembled as rods or filaments, but several types of virus including the influenza and para-influenza groups enclose the nucleocapsid in an envelope. See section 5.6.

3.1 Combined symmetry forms in viruses

In the electron microscope many of the large and more complex virus particles show evidence for structural organization where both icosahedral and helical symmetry have been combined in the same virion. The most striking examples are the tailed bacteriophages (Figs. 4–1 and 4–2). There are other groups of complex viruses including the rhabdoviruses shown in Figs. 5–11 and 5–12 and the poxviruses (Figs.

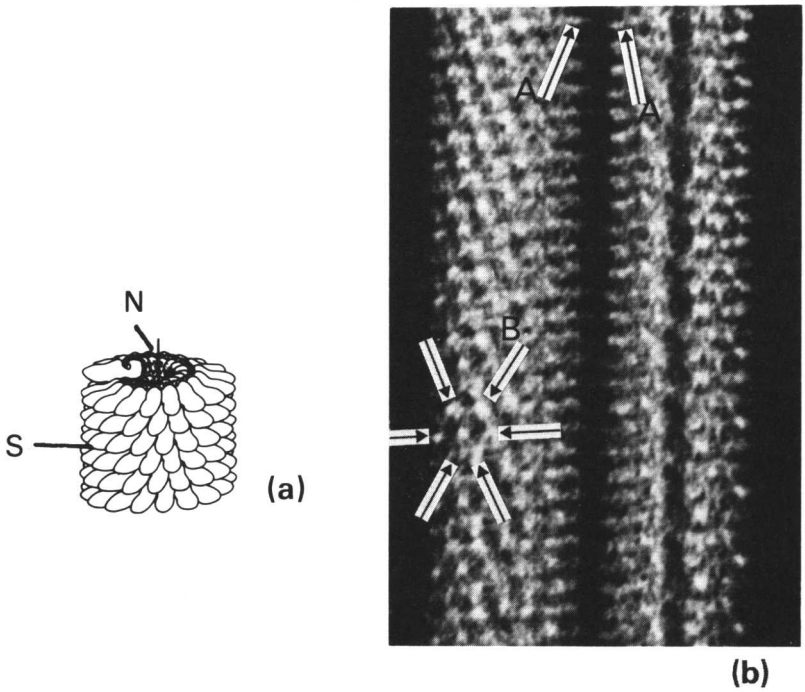

Fig. 3–3 (**a**) Drawing showing part of a tobacco mosaic virus (TMV) rod based on X-ray diffraction and biochemical data. The individual protein structure units (**S**) are arranged in helical symmetry, generating the primary and secondary helical patterns. There are approximately $16\frac{1}{3}$ structure units in each primary turn of the helix with a central structure unit surrounded by six neighbours. The nucleic acid (**N**) is RNA and deeply embedded in the protein (shown exposed for diagrammatic purposes). The diameter of the TMV rod is about 17.0 nm with the RNA located at a radius of 4 nm. A central axial hole of 4 nm diameter extends throughout the length of the TMV rod. (By permission of D. L. D. Caspar.) (**b**) Electron micrograph of two TMV rods arranged in parallel. The micrograph was subjected to image processing to reinforce their regular structural features. The rod on the left shows the same basic features as the drawing in (**a**). The 2.3 nm helical pitch (**A**) is clearly resolved together with the structure units (**B**). Note the central structure units surrounded by six neighbours (arrows **B**), together with the primary and secondary helical patterns. The rod on the right shows the axial central hole following penetration by electron dense stain. (From HORNE, R. W., HARNDEN, J. M., and MARKHAM, R. (1976). *J. Gen. Virology*, **31**, 261–9.)