IV.

Morphology of Viruses

The chemical constituents described in the previous chapter are found in particles of diverse size and shape in the various viruses isolable from animals, bacteria, plants, and fungi. Despite the diversity of size and shape of different viruses, the size and shape of any one virus tend to be much more uniform than do the cells of a bacterium. This uniformity is reflected in the fact that many viruses can be crystallized whereas bacteria cannot. Some examples of virus crystals are shown in Figure 30. Note that a single virus crystal contains millions of virus particles as is nicely illustrated in the electron micrograph obtained by Steere and Williams (1953) of a partially dissolved crystal of tobacco mosaic virus. Thus, although a simple virus particle may consist of hundreds of molecules of protein and one or more molecules of nucleic acid, large populations of these particles often behave as though they were just molecules, crystallization of particles being one manifestation of this characteristic (behavior of virus particles in hydrodynamic tests such as electrophoresis or sedimentation is also molecular in character).

Each virus has a characteristic size and shape. The range in size for viruses as a group is from about 20 nm in diameter for minute virus of mice to about 300 nm for a poxvirus (some elongated plant and bacterial viruses exceed this upper limit in one dimension; for example, beet yellows virus is about 10 × 1,250 nm).

Few distinctive shapes have been observed among viruses, and most viruses fall into one or another of three general groups characterized by (1) spheroidal particles (also called spherical or isometric particles); (2) elongated particles; and (3) combination particles, such as a tailed bacteriophage that may have a spheroidal head and an elongated tail. The sizes and shapes of viral particles in some distinctive groups of algal, animal, bacterial, insect, and plant viruses are given in Tables 30 to 34.

Although some attempt was made in these tables to group viruses according to recommendations of international committees concerned with virus classification and nomenclature, the purpose of the tables is not to deal with virus classification, but rather to illustrate the distribution of sizes and shapes among distinguishable classes of viruses. In order to treat viruses in groups, the dimensions assigned must necessarily encompass the

C. A. Knight, *Chemistry of Viruses*

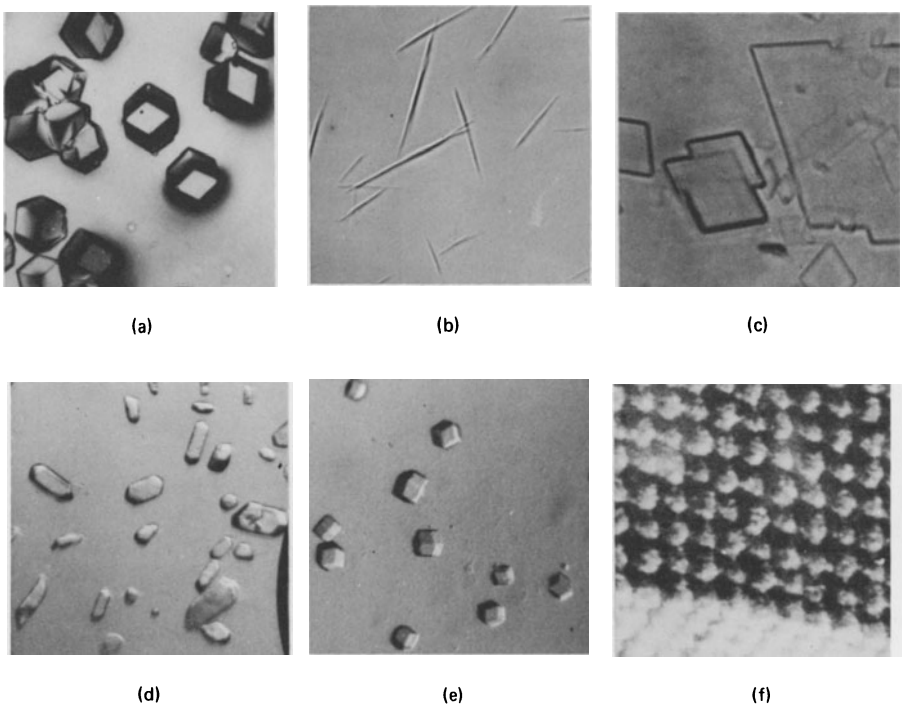

Fig. 30. Crystals of some viruses. *a*. Tomato bushy stunt virus; *b*. tobacco mosaic virus; *c*. Southern bean mosaic virus; *d*. poliovirus; *e*. polyoma virus; *f*. electron micrograph of a portion of a crystal of tobacco necrosis virus showing orderly array of virus particles. (*a* and *b*, courtesy W. M. Stanley; *d*, courtesy F. L. Schaffer; *e*, courtesy W. T. Murakami; and *f*, courtesy R. W. G. Wyckoff.)

range found in the group and thus suffer in precision for individual viruses. However, when precision in dimensions is required, it can be obtained from the references accompanying each table. Another caveat about virus dimensions is that some viruses are more plastic than others; enveloped viruses are most apt to be pleomorphic and to exhibit a range of sizes and shapes. Some of these points will be illustrated in electron micrographs of different viruses where it will also be evident that viruses occur in many sizes but in relatively few shapes.

Evidence concerning the size and shape of virus particles was obtained by indirect methods for some years prior to the common availability of the electron microscope. Some of these methods are still very useful. Thus, estimates of size can be obtained by ultrafiltration, diffusion measurements, gel chromatography, and light scattering, while indications of shape are readily obtained by flow birefringence or viscosity measurements; density

Table 30. Sizes and Shapes of Some Blue-Green Algal Viruses.[a]

Virus	Diameter or Dimensions (nm)	Shape
Anacystis, Synechococcus[b] (AS) AS-1	Head 90 Tail 23 × 244	Spheroidal head and elongated tail
Lyngbya, Plectonema, Phormidium (LPP) LPP-1, LPP-2	Head 59 Tail 15 × 20	Spheroidal head and short tail
Nostoc (N) N-1	Head 55 Tail 16 × 110	Spheroidal head and elongated tail
Synechococcus, Microcystis (SM) SM-1	88	Spheroidal with collar and possibly a very short tail

[a]Compiled from Brown 1972.

[b]These viruses are named according to the algal genera the viruses infect; hence, the names in the table are generic names of some susceptible blue-green algae. The algal viruses contain linear, double-stranded DNA. (See also Padan and Shilo 1973.)

alone, or a composite indication of size, shape , and density can be determined by various centrifugation techniques. Applications of some of these methods were described in the section on Purification of Viruses.[1]

The most versatile and direct method for determining the size and structure of virus particles is by electron microscopy. Many techniques are available that enhance the usefulness of the electron microscope beyond its ability to resolve objects down to about the 1 nm level in contrast to the approximately 200 nm resolving power of the light microscope. Some of these techniques increase contrast between virus particles and the plastic film of the microscope mount, some minimize the tendency of particles to collapse when exposed to osmotic and surface tension forces, and others limit the destructive effects of beams of electrons used to illuminate the field under examination. For descriptions of these methods and their applications, see Kay (1961), Huxley and Klug (1971), Dalton and Haguenau (1973), Williams and Fisher (1974); for reviews, see Horne (1967) and Milne (1972).

The development of electron microscopy, coupled with chemical and physical analyses, revealed various features of virus particles that might be called ultrastructural details. Many such structural components have been given names (Caspar et al. 1962; Lwoff and Tournier 1966); the commoner terms and their synonyms will be briefly presented here.

[1]Detailed descriptions can be obtained in such works as *Methods in Virology*, Vol. 2, K. Maramorosch and H. Koprowski, editors, New York: Academic Press (1967).

Table 31. Sizes and Shapes of Some Diverse Groups of Animal Viruses.[a]

Virus	Diameter or Dimensions (nm)	Shape
A. DNA-Containing Vertebrate Viruses		
Adenoviruses:	70–90	Spheroidal with projecting fibers
Avian adenoviruses		
Gallus-adeno-like (GAL)		
Chicken-embryo-lethal-orphan (CELO)		
Bovine adenoviruses		
Canine adenoviruses		
Infectious canine hepatitis virus (ICH)		
Human adenoviruses		
31 serological types		
Murine adenoviruses		
Ovine adenoviruses		
(may be same as bovine strains)		
Porcine adenoviruses		
Simian adenoviruses		
Herpesviruses:	100–150	Spheroidal with envelope
Group A		
B virus of monkeys, equine abortion, equine respiratory disease, feline rhinotracheitis, herpes simplex (types 1 and 2), infectious bovine rhinotracheitis, infectious laryngotracheitis, owl monkey herpes, marmoset herpes, squirrel monkey herpes		
Group B		
Cytomegalovirus, varicella-zoster		
Group C		
Burkitt lymphoma, herpesvirus ateles, herpesvirus saimiri,		

herpesvirus sylvilagus, Lucké frog tumor, Marek's disease of chickens		
Papovaviruses	43–53	Spheroidal
K virus of mice		
Papilloma viruses		
Bovine, canine, rabbit, human (wart)		
Polyoma of mice		
Vaccuolating viruses: rabbit, simian (SV40)		
Parvoviruses (picodnaviruses)	18–22	Spheroidal
Adeno-associated viruses (AAV)		
Hamster osteolytic viruses		
Latent rat viruses (Kilham rat virus (RV), X14, H-1, H-3)		
Minute virus of mice (MVM)		
Poxviruses	230 × 300	Brick shaped with core, lateral bodies, outer membrane with whorled surface filaments
True poxviruses		
Vaccinia-variola group		
Alastrim, cowpox, ectromelia, monkeypox, rabbitpox, vaccinia, variola (smallpox)		
Fibroma-myxoma group		
Hare fibroma, rabbit fibroma, rabbit myxoma, squirrel fibroma		
Birdpox group		
Canarypox, fowlpox, pigeonpox, turkeypox		
Sheeppox group		
Goatpox, lumpyskin disease, sheeppox		
Orf group		
Orf, bovine papular stomatitis, pseudocowpox		
Ungrouped poxviruses	150 × 200	Ovoid, with surface filaments
Molluscum contagiosum, swinepox, Yaba monkey tumor		

Table 31. Sizes and Shapes of Some Diverse Groups of Animal Viruses.[a] (cont.)

Virus	Diameter or Dimensions (nm)	Shape
B. RNA-Containing Vertebrate Viruses		
Arenaviruses Lassa, Lymphocytic choriomeningitis (LCM) Tacaribe hemorrhagic fever (several viruses)	50–150	Spheroidal with envelope
Coronaviruses Avian infectious bronchitis (IBV), several human respiratory viruses, mouse hepatitis, and rat pneumonotropic	70–120	Spheroidal with envelope
Diplornaviruses Orbiviruses (bluetongue group) African horse sickness, bluetongue, Changuinola, Chenuda, Colorado tick fever, epizootic hemorrhagic disease of deer, Eubenagee, Irituia, Palyam, simian virus SA-11, Tribec, Wad Medani	60–80	Spheroidal with inner and outer capsids
Reoviruses Avian reoviruses (5 serological types) Mammalian reoviruses (3 serological types)		

Myxoviruses	100–350	Spheroidal with envelope and projecting spikes; filamentous forms are also common
Metamyxoviruses		
pneumonia virus of mice		
respiratory syncytial (RS)		
Orthomyxoviruses	90–120	
Equine influenza, fowl plague, human influenza types A, B, and C, swine influenza		
Paramyxoviruses	120–450	
Mumps, Newcastle disease of chickens, parainfluenza (cattle, man, mice) types 1-4, simian virus 5 (SV5)		
Pseudomyxoviruses	120–300	
Canine distemper, measles, rinderpest		
Oncornaviruses	About 100	Spheroidal with envelope
Leukosis (leukemia) viruses		
Avian leukosis viruses (ALV)		
Lymphomatosis (RPL-12 and other strains), erythroblastosis (AEV), MC29, myeloblastosis (AMV), Rous-associated viruses (RAV-1, RAV-2, etc.)		
Murine leukemia viruses (MLV)		
Friend, Graffi, Gross, Kirsten, Moloney, Rauscher, etc.		
Mammary tumor viruses (MTV)		
Bittner, human (?)		
Miscellaneous oncornaviruses		
Feline, hamster, human (?), monkey, rat, reptile, etc.		
Sarcoma viruses		
Avian sarcoma viruses		
Fujinama sarcoma,		
Rous sarcoma (RSV) (several strains)		
Murine sarcoma viruses		
Harvey, Kirsten, Maloney		

Table 31. Sizes and Shapes of Some Diverse Groups of Animal Viruses.[a] (cont.)

Virus	Diameter or Dimensions (nm)	Shape
Picornaviruses	20–30	Spheroidal
Enteroviruses		
Encephalomyocarditis		
Columbia SK, encephalomyocarditis (EMC), mengo, mouse Elberfeld (ME)		
Human		
Coxsackie A, Coxsackie B, ECHO, polio		
Mouse encephalomyelitis		
Theiler's virus		
Simian enteroviruses (multiple serotypes)		
Rhinoviruses		
Human, other animals		
Unclassified picornaviruses		
Foot-and-mouth disease (FMDV), vesicular exanthema of swine (VE)		
Rhabdoviruses	75 × 130–230	Bullet-shaped or bacilliform with envelope
Bovine ephemeral fever, Chandipura, Flanders-Hart Park, Kern Canyon, Lagos, Marbur, Oregon sockeye disease, rabies, vesicular stomatitis, and others		
Togaviruses	40–60 for alpha- and flavoviruses and 100 for Bunyamuera supergroup	Spheroidal with envelope
Alphaviruses (Arbovirus Group A)		
Bebaru, Chikungunga, eastern equine encephalitis (EEE), Mayaro, Mucambo, O'nyong nyong, Pixuna, Ross River, Semiliki forest, Sindbis, Venezuelan equine encephalitis (VEE), Western equine encephalitis (WEE)		

Flavoviruses (Arbovirus Group b)
Dengue, diphasic meningoencephalitis, Japanese encephalitis, louping ill, Powassan, St. Louis encephalitis, West Nile, yellow fever
Bunyaviruses (Bunyamwera Supergroup)
Bunyamwera, California encephalitis, Inkoo
Miscellaneous togaviruses
Lactic dehydrogenase (LDH) of mice, phlebotomous fever, rubella

[a]Adapted from Melnick 1971, 1972. See also Joklik and Smith 1972, pp. 747–754.

Table 32. Sizes and Shapes of Some Bacterial Viruses.[a]

Virus	Diameter or Dimensions, nm		Shape
	Head	Tail	
A. Phages with contractile tails:			
Alcaligenes faecalis A6	90	16 × 110	Spheroidal head and rodlike tails ("spheroidal" includes oblong, octahedral, icosahedral, etc.)
Bacillus subtilis SPO1	90	30 × 210	
Escherichia coli E1	75	17 × 210	
Escherichia coli T2, T4, T6	65 × 95	25 × 110	
Lactobacillus 206	72	16 × 138	
Myxococcus xanthus MX1	75	25 × 100	
Proteus hauseri 78	61	16 × 89	
Pseudomonas aeruginosa PB1	75	20 × 140	
B. Phages with noncontractile tails:			
Escherichia coli lambda	54	10 × 150	Spheroidal head and rod-like or flexuous tail
Escherichia coli T1	50	10 × 150	
Escherichia coli T3, T7	60	10 × 15	
Escherichia coli T5	65	10 × 170	

Table 32. Sizes and Shapes of Some Bacterial Viruses.[a](cont.)

	Virus	Diameter or Dimensions, nm		Shape
	Pseudomonas Pc	65	10 × 160	
	Staphylococcus 6	40 × 92	10 × 300	
	Streptococcus 3ML	40 × 55	9 × 100	
	Typhoid 1	75	9 × 180	
	Typhoid S1 BL	50	10 × 130	
C.	Tailless phages:			
	1. With apical structures Escherichia coli α3, ØX174, ØR, S13	27		Spheroidal
	2. With apical structures but enveloped *Pseudomonas* PM2	60		Spheroidal with envelope
	3. Without apical structures[b]			Spheroidal
	Caulobacter crescentus Cb23r	22		
	Escherichia coli f2, fr, MS2, Qβ, R17	24		
	Pseudomonas aeruginosa 7s	25		
D.	Filamentous phages:			
	Escherichia coli f1, fd, M13	6 × 800		Filamentous
	Pseudomonas aeruginosa Pf	6 × 1300		
	Salmonella typhimurium If1, If2	6 × 1300		

[a]Compiled from Bradley and Kay 1960; Bradley 1971; Joklik and Smith 1972, p. 829. The phages are listed by name of host bacterium followed by designation of phage.

[b]The phages of group C3 contain RNA; all of the others are DNA phages.

Table 33. The Sizes and Shapes of Some Insect Viruses.[a]

Virus	Diameter or Dimensions, nm	Shape
A. Occluded viruses (occur in inclusion bodies):		
1. Granulosis viruses		Rodlike (occur in inclusions called granules or capsules)
Armyworm	62 × 412	
Codling moth	51 × 314	
Spruce budworm	40 × 270	
2. Polyhedrosis viruses		
Nuclear		Rodlike (occur in inclusions called polyhedral bodies)
Gypsy moth	18 × 280	
Silkworm	40 × 280	
Western oak looper	62 × 332	
Cytoplasmic		Spheroidal with surface projections (occur in inclusions called polyhedral bodies)
Monarch butterfly	67	
Silkworm (CPV)	69	
Spruce budworm	70	
3. Insect poxviruses		Brick shaped with core and lateral body (occur in inclusions called spherules)
Amsacta pox	250 × 350	
Melolontha pox	250 × 400	
4. Beetle viruses		
Melolontha (cockchaffer) spindle disease	250 × 370	Ovoid (occur in spindle-shaped or ovoid inclusions)
B. Nonoccluded viruses:		
1. Iridescent viruses Chilo, Sericesthis, and *Tipula iridescent* viruses (CIV, SIV, TIV)	150	Spheroidal

Table 33. The Sizes and Shapes of Some Insect Viruses.[a](cont.)

Virus	Diameter or Dimensions, nm	Shape
2. Miscellaneous viruses		
Acute bee paralysis	28	Spheroidal
Antherea	50	Spheroidal
Densonucleosis virus of Galleria (DNV)	20	Spheroidal
Sac brood of bees	28	Spheroidal
Sigma virus of *Drosophila*	70 × 200	Bullet-shaped with envelope

[a]Compiled from Smith 1967; Smith 1971; Bellet 1968; Bergoin and Dales 1971; Vago and Bergoin 1968; Kurstak 1972. It should be noted that there are numerous plant viruses, especially of the rhabdovirus type, that multiply in both plants and insects and are not listed here (see Knudson 1973).
The cytoplasmic polyhedrosis viruses contain double-stranded RNA and some of the miscellaneous nonoccluded viruses such as that of sac brood of bees contain RNA; all of the rest appear to contain DNA.

Table 34. Sizes and Shapes of Some Diverse Groups of Plant Viruses.[a]

Virus	Diameter or Dimensions, nm	Shape
Alfalfa (lucerne) mosaic	18 × 18; 18 × 36; 18 × 48; 18 × 58	Pleomorphic: 3 bacilliform particles and 1 spheroidal particle
Beet yellows	10 × 1250	Flexuous rods
Brome mosaic	25	Spheroidal
Also: broad bean mottle virus, cowpea chlorotic mottle		

Carnation latent Also: Cactus 2, chrysanthemum B, pea streak, potato virus M, potato virus S, red clover vein mosaic	15 × 620–700	Bent rods
Cauliflower mosaic Also: Carnation etched ring, dahlia mosaic	50	Spheroidal
Clover wound tumor Also: Maize rough dwarf, rice dwarf	70	Spheroidal
Cowpea mosaic Also: Bean pod mottle, broad bean stain, radish mosaic, red clover mosaic, true broad bean mosaic	30	Spheroidal
Cucumber mosaic Also: Cucumber yellow mosaic, tomato aspermy	30	Spheroidal
Pea enation mosaic	28	Spheroidal
Potato virus X Also: Cactus X, clover yellow mosaic, hydrangea ringspot, white clover mosaic	13 × 480–540	Flexuous rods
Potato virus Y Also: Bean common mosaic, bean yellow mosaic, beet mosaic, clover yellow vein, Columbian datura, cowpea aphid-borne mosaic, henbane mosaic, pea mosaic, potato virus A, soybean mosaic, tobacco etch, watermelon mosaic (S. Africa)	15 × 730–790	Flexuous rods

Table 34. Sizes and Shapes of Some Diverse Groups of Plant Viruses.[a](cont.)

Virus	Diameter or Dimensions, nm	Shape
Potato yellow dwarf Also: Lettuce necrotic yellows, eggplant mottled dwarf, maize mosaic, Russian winter wheat mosaic, sowthistle yellow vein	50–100 × 200–300	Bacilliform with lipid containing envelope
Prunus necrotic ringspot Also: Apple mosaic, rose mosaic	25	Spheroidal
Southern bean mosaic	30	Spheroidal
Tobacco mosaic Also: Cucumber green mottle mosaic, cucumber yellow mottle mosaic, odontoglossum ringspot, ribgrass mosaic, Sammons opuntia, sunn hemp mosaic, tomato mosaic	18 × 300	Tubular rods
Tobacco necrosis	28	Spheroidal
Tobacco rattle Also: Pea early browning	22 × 50–102 and 22 × 170–210	Tubular rods of two characteristic lengths

Tobacco ringspot Also: Arabis mosaic, grapevine fanleaf, raspberry ringspot, strawberry latent ringspot, tomato black ring, tomato ringspot	30	Spheroidal
Tomato bushy stunt Also: Artichoke mottle crinkle, carnation Italian ringspot, pelargonium leaf curl, petunia asteroid mosaic	30	Spheroidal
Tomato spotted wilt	70–80	Spheroidal
Turnip yellow mosaic Also: Andean potato latent, belladonna mottle, cacao yellow mosaic, dulcamara mottle, eggplant mosaic, ononis yellow mosaic, wild cucumber mosaic	30	Spheroidal

[a]Examples were taken mainly from Harrison et al. 1971 in which a group is defined as "a collection of viruses and/or virus strains, each of which shares with the type member all or nearly all the main characteristics of the group." All of the viruses listed contain single-stranded RNA except the clover wound tumor group, which has double-stranded RNA, and the cauliflower mosaic group has double-stranded DNA instead of RNA. The above list is an alphabetic arrangement according to type member of a group. For additional details about listed viruses see the semiannual compilations, *Descriptions of Plant Viruses*, from 1970 on, Gibbs et al. eds. These compilations are issued jointly by the Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, England, and the Association of Applied Biologists. Orders should be addressed to Central Sales Branch, Commonwealth Agricultural Bureaux, Farnham Royal, Slough SL 2 3BN, England.

The mature (structurally complete), potentially infectious virus particle is called a *virion*. Virus, or virus particle, are synonyms, although in one usage the term "virus" embraces all phases of the viral life cycle rather than just the mature virus particle. *Capsid* is a term given to the protein built around and closely associated with the viral nucleic acid, the combination of the two being called *nucleocapsid, nucleoprotein* (*NP*), or *core*. Synonyms for capsid are *protein coat* and *protein shell*. *Structure units* are the identical protein molecules that make up the capsid; they are also known as *protein subunits*. *Capsomers* are the capsid substructures distinguishable in the electron microscope. They may be individual protein subunits or more often represent small clusters (for example, two, five, or six) of subunits; capsomers are also called *morphologic units*. Viruses that mature at cell membranes may acquire a structure consisting of lipid, protein, and carbohydrate that surrounds and encloses the nucleocapsid, and hence is called *envelope* (*peplos* has also been suggested for this structure but has not been widely adopted). Projections from the surface of a virus particle, especially from the surface of enveloped viruses, are called *spikes* and occasionally *peplomers*. A schematic diagram of three types of virus particles showing some of these structural features is given in Figure 31.

A basic feature of virus morphology is that a virus particle is in many instances composed of numerous identical protein subunits and one or a few molecules of nucleic acid. Also, the shape of a virus particle is usually determined by the virus protein since this comprises most of the mass of the particle, and the configuration and interactions of protein subunits are essentially fixed by the amino acid sequences they possess. A combination of the data issuing from chemical, x-ray, and electron microscopic analyses with principles of symmetry from solid geometry and model building has led to the conclusion that there are two basic designs generally used in nature in the fabrication of virus particles from protein subunits: helical tubes and icosahedral shells (see Horne and Wildy 1961; Caspar and Klug 1962).

In a particle showing helical symmetry, the protein subunits are arranged in a regular helical array perpendicular to the long axis of a particle. This arrangement may result in a tubular structure such as in the tobacco mosaic virus particle (see model, Figure 18 and 7) or a flexuous strand as in the shell of elongated plant and bacterial viruses (for example, potato virus X and coliphage fd, Figure 35) or in the elongated but folded nucleoprotein components of animal viruses such as influenza, vesicular stomatitis, and Sendai viruses (Figure 34A). (Note that the helical nucleoprotein of influenza virus is enclosed in a spheroidal envelope which, though made of repeating units, cannot be readily classified in terms of symmetry.)

Icosahedral symmetry (a form of cubic symmetry) exhibited by many spheroidal virus particles requires that there be specific axes of symmetry (five-, three-, and twofold) about which the particles can be rotated to give a series of identical appearances.

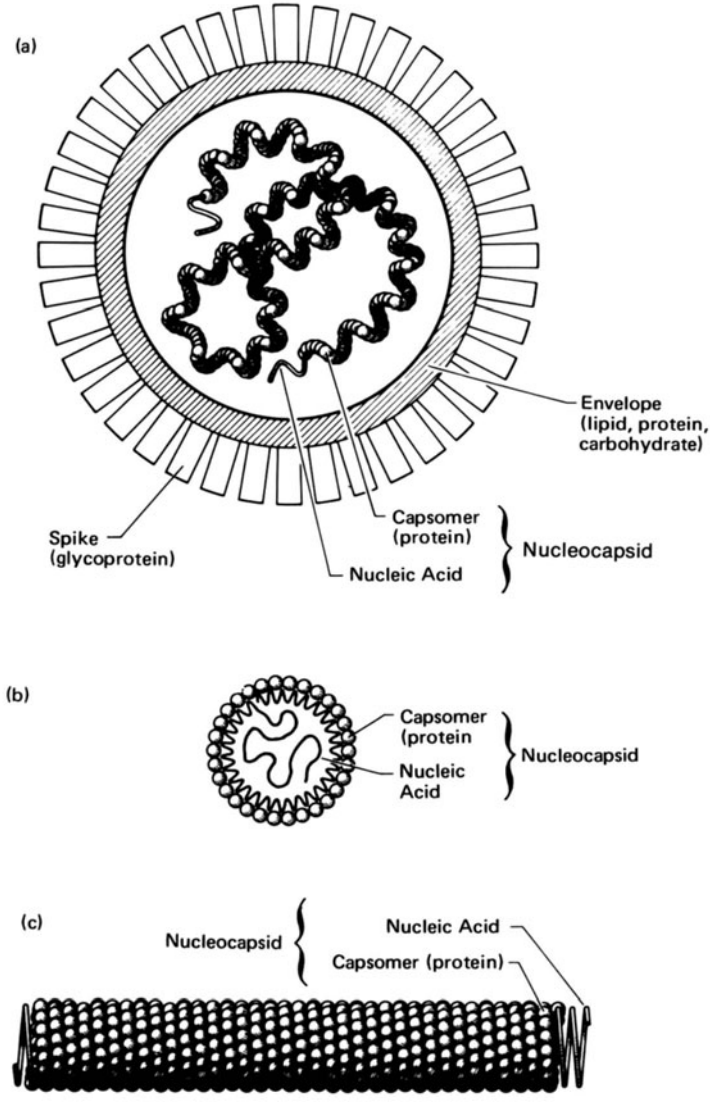

Fig. 31. Schematic diagram of three types of virions. *a*. Enveloped virion with helical nucleocapsid. *b*. Spheroidal virion. *c*. Elongated virion.

It would be an oversimplification to state that the structures of virus particles exhibit either helical symmetry or icosahedral symmetry, for some virus particles have very complex structures. Nevertheless, it is remarkable that the particle structures of many viruses can be interpreted in terms of helical and icosahedral symmetries. Some viruses exhibit both, as for ex-

ample, a tailed bacteriophage whose head may show icosahedral symmetry and the tail, helical symmetry. In terms of icosahedral symmetry it can be predicted that spheroidal viruses will have specific numbers of morphologic units. Some examples of the classes according to number of morphologic units, and some viruses possibly illustrating the classes, are given in Table 35. The numbers of protein subunits are also given in the table as a reminder that the units visualized in the electron microscope (morphologic units) usually consist of more than one protein subunit. In the Caspar and Klug concept of icosahedral viruses, the protein subunits may be thought to occur in groups of five (pentamers) and six (hexamers), as the

Table 35. Possible Numbers of Morphologic Units and Subunits in Virus Particles Having Icosahedral Symmetry.[a,b]

No. of Morphologic Units	No. of Subunits	Grouping of Subunits in Forming Morphologic Units[c]	Virus Example
12	60	12 pentamers	Coliphage ØX174
32	180	12 pentamers 20 hexamers	Broad bean mottle, cowpea chlorotic mottle, cucumber mosaic, turnip yellow mosaic
42	240	12 pentamers 30 hexamers	Arabis mosaic, tobacco ringspot
72	420	12 pentamers 60 hexamers	Human wart, polyoma, simian virus 40, Shope papilloma
90	180	90 dimers	Tomato bushy stunt, turnip crinkle
92	540	12 pentamers 80 hexamers	Reovirus, wound tumor
162	960	12 pentamers 150 hexamers	Herpes simplex, varicella
252	1,500	12 pentamers 240 hexamers	Adenovirus, infectious canine hepatitis

[a]From Knight 1974.

[b]There are classes of icosahedral particles other than those listed here, but they were omitted for lack of virus examples to illustrate them. See Caspar and Klug (1972) for a detailed discussion.

[c]These groupings of subunits are conceptual and may or may not coincide with the actual situation. For example, coliphage ØX174 seems to have four different protein components rather than 60 copies of one, and the precise numbers and morphologic arrangement of the four proteins remain to be worked out. Similarly, adenovirus has several different protein components, of which the major coat constituent, the hexon, probably consists of three polypeptides, which, moreover, are not identical.

examples in Table 35 indicate. The number of subunits per particle is 60 or some multiple of 60.

Finally, it should be noted that while the concepts of symmetry can be very important in studies of virus fine structure, molecular structures, as Caspar and Klug indicated, are not built to conform to exact mathematical concepts, but rather to satisfy the condition that the system be in a minimum energy configuration. Moreover, with modern techniques of electron microscopy, one can obtain considerable information about virus structures without any knowledge of symmetry in the mathematical sense.

In electron microscopy of viruses, contrast between particle and mount was greatly enhanced by introduction of a shadowing technique (Williams and Wyckoff 1945) in which the particles are coated obliquely with metal vapors in vacuo. This technique is tremendously useful in enhancing the contrast between virus particles and the medium on which the particles are supported, but the metal coating often obscures surface details. An exception is the Shope papilloma virus, shadowed particles of which were observed to show regular arrays of knobs (Figure 32) (Williams 1953b). This appears to represent the first direct observation of morphologic units, each of which is now thought to be composed of five or six protein subunits (see Table 35).

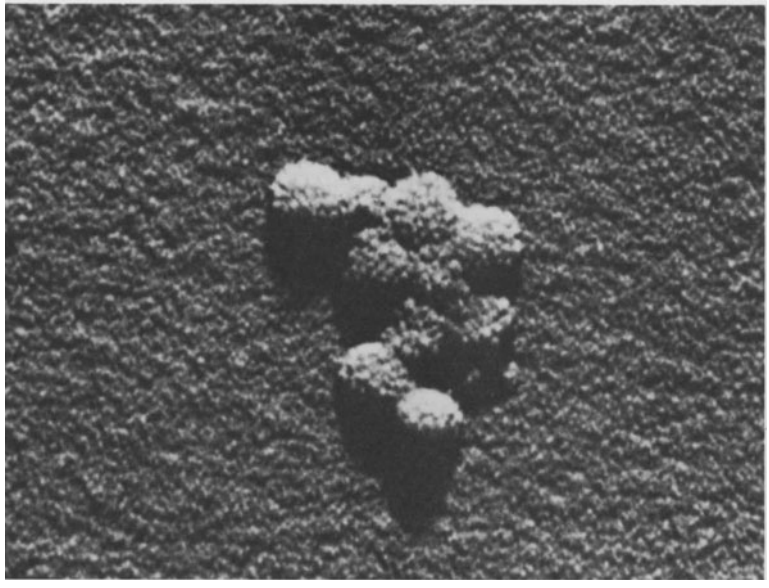

Fig. 32. Micrograph of a cluster of air-dried, uranium-shadowed particles of Shope papilloma virus showing regularly arranged surface knobs. (From Williams 1953b.)

A major advance in visualizing morphologic units as well as other structural features of virus particles occurred with Huxley's (1957) demonstration of the central hole in the TMV particle with a "negative staining" technique. This method, elaborated by Brenner and Horne (1959), was subsequently used extensively by Horne and associates (see Horne 1962) and is now universally employed. It may be briefly described as follows:

A 2 percent solution of phosphotungstic acid (PTA) is brought to neutrality or slightly above by the addition of N KOH. Equal volumes of virus (usually about 10–100 μg/ml in water or ammonium acetate) and PTA are mixed and transferred to a carbonized electron microscope grid from which much of the applied drop is removed with a small strip of filter paper. The grid is allowed to dry and then is examined in the electron microscope. Another method for applying the virus-phosphotungstate mixture is by spraying from an atomizer, giving a very fine mist. The advantage of this technique is that one can get isolated fields (spray droplets), the particles of which are more or less representative of the whole population and are contained within a single field. There are other variations of the technique, including washing of the mounts after application of the virus or virus-phosphotungstate mixture in order to remove excessive salts or small molecules, the virus generally adhering more firmly to the mount than the smaller molecules. Also, uranyl acetate or uranyl formate is sometimes substituted for phosphotungstate, especially if there is any evidence that the virus is unstable in phosphotungstate as is, for example, alfalfa mosaic virus (Gibbs et al. 1963).

With negative staining, the PTA, under the usual conditions, does not adhere specifically to the virus particles as it would in positive staining (which can be done under appropriate conditions). Rather, as the mount dries, the PTA drains down the virus particles and deposits on the particles and on the supporting mount in such a way as to reflect the topography and internal hollow regions of the particles. The micrographs presented here to illustrate the structure of different viruses were made by the negative staining technique.

A. Nonenveloped Spheroidal Viruses

Some spheroidal plant, bacterial, and animal viruses of various sizes are illustrated in Figure 33. Morphologic units are discernible in most of the particles, in some more clearly than in others. The supposed numbers of such units are indicated in Table 35. The viruses illustrated in Figure 33, as is also the case with those shown in Figures 34 to 37, are representative of dozens of other viruses (see Tables 30 to 34 for a partial listing).

Comparison of the particles shown for coliphages ØX174 and Qβ illustrates an interesting difference between these viruses. Both have spheroi-

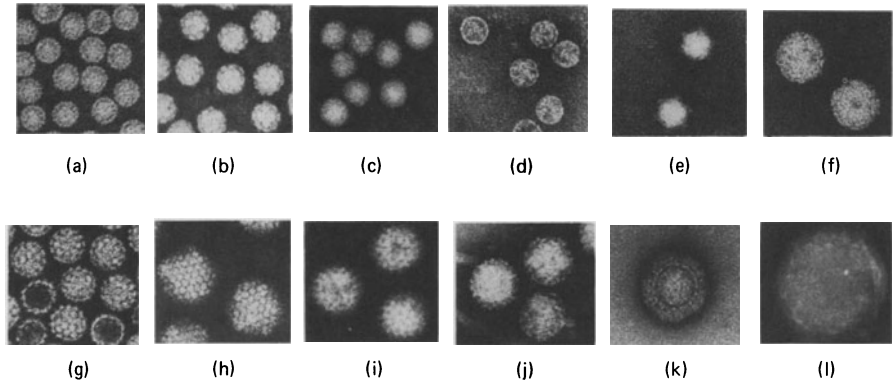

Fig. 33. Some nonenveloped spheroidal viruses. *a*. brome mosaic virus; *b*. turnip yellow mosaic virus; *c*. poliovirus; *d*. Qβ coliphage; *e*. ØX174 coliphage; *f*. cauliflower mosaic virus; *g*. Shope rabbit papilloma virus; *h*. adenovirus-5; *i*. wound tumor virus of sweet clover; *j*. cytoplasmic polyhedrosis virus of the silkworm, *k*. reovirus; *l*. *Tipula iridescent* virus. The virions in the top row are about 30 nm in diameter except for that of cauliflower mosaic, which is about 50 nm; the virions shown on the bottom row range about 50–130 nm in diameter. All mounts were prepared by the negative staining technique (see text). Note the morphologic units exhibited by some virions and especially the apical knobs on the ØX174 particles. (Courtesy R. C. Williams and H. W. Fisher.)

dal particles, but apical knobs are discernible on the ØX174 particles and not in those of Qβ. One or more of the knobs on the ØX174 particles may serve in the specific attachment of this and similar viruses to bacterial cells susceptible to infection by these phages.

Adenoviruses are among the larger viruses (about 80 nm in diameter) and consequently the faces of its icosahedral particles are more clearly evident than in smaller viruses of this shape. The particles have been studied extensively, and it is known that there are 252 morphologic units in the coat protein; these fall into two structural groups termed hexons and pentons. There are 240 hexons (each hexon consists of six protein subunits in a regular cluster) comprising most of the protein coat (capsid) of the virion. The hexons are polygonal discs about 7–8 nm in diameter with a central hole about 2.5 nm across (See Figure 33A) and each hexon is bounded by six other morphologic units. The 12 pentons are situated at the 12 vertices of the icosahedron and each is bounded by five morphologic units. The pentons serve as base structures to which fibers, called penton fibers, are attached. Each penton fiber is about 2 × 20 nm and terminates in a spherical knob about 4 nm in diameter. These structures are often invisible in the electron dense PTA medium employed in negative staining, but in areas where the PTA matrix is less dense they can be discerned as shown with one particle in Figure 33A. The penton fibers are important in the serologic and hemagglutinating activities of adenoviruses and may also

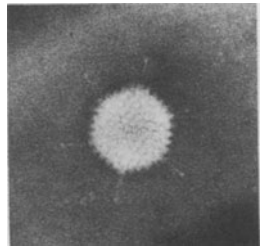

Fig. 33A. An adenovirus virion showing some hexon and penton units (Courtesy R. C. Williams and H. W. Fisher.)

serve as attachment organs in initiating the process of infection (Horne 1973).

It is not uncommon to find particles in preparations of various viruses that, although otherwise closely resembling intact particles in size and shape, are lacking in nucleic acid. Such empty particles exhibit dark centers on electron micrographs, presumably reflecting the ability of PTA to flow readily through empty viral shells and puddle beneath the particles in larger amounts than under complete particles. This is illustrated in the micrograph of Shope papilloma virus in Figure 33. It should be noted in this connection that phosphotungstate may cause a proportion of initially full particles to leak out their nucleic acid (Milne 1972), since a much smaller percentage of empty particles is observed with sensitive viruses when uranyl acetate is employed as the negative stain.

The particles of diplorna viruses such as reovirus are distinctive in containing segmented, double-stranded RNA, as well as for having two protein shells, an outer and an inner one. The morphologic units of both shells are arranged according to icosahedral symmetry. The outer shell appears as a ring in the micrograph of reovirus shown in Figure 33. The outer shell can be digested away with chymotrypsin to leave the inner nucleocapsid, or "core." However, it is not yet clear whether other diplornaviruses have double capsids. The particles of wound tumor virus of sweet clover (WTV) (Figure 33) and of silkworm cytoplasmic polyhedrosis virus (CPV) approximate the size of reovirus cores rather than whole particles and are also similar to cores in possessing RNA transcriptase activity (Lewandowski and Traynor 1972). Of course it is possible that the outer capsids of WTV and of CPV are more readily lost in isolating these viruses than is that of reovirus. This is especially a possibility with CPV, which is usually extracted from polyhedral bodies at rather high pH.

The particles of the iridescent insect viruses (the term "iridescent" comes from the fact that diseased tissues as well as gelatinous pellets of

purified virus obtained by centrifugation are iridescent when examined by reflected white light) are the largest presently known nonenveloped viruses; they have a diameter of about 150 nm and are clearly icosahedrons (Williams and Smith 1958). Negatively stained particles, such as those of *Tipula iridescent* virus shown in Figure 33, exhibit a hexagonal outline on micrographs and the protein coat appears as a membranous one- or two-layer structure.

A general point can be made here concerning the relationship between protein and nucleic acid in viruses. There is no evidence for covalent linkage of these substances in any type of virus; nevertheless, the secondary attractions between protein and nucleic acid tend to result in specific configurations. With respect to spheroidal viruses, the nucleic acid is not just randomly packed in a protein shell. For example, x-ray analyses made on turnip yellow mosaic virus (Klug et al. 1966) indicate a regular interlacing of the nucleic acid with the protein subunits far enough below the surface of the particle to protect the nucleic acid from outside degradative agents. The crude sketch of Figure 31 is intended to suggest this relationship as opposed to the simple bag-of-nucleic-acid concept. A similar structure has been deduced for broad bean mottle virus (Finch and Klug 1967). However, specific details of protein-nucleic acid association for spheroidal viruses, as well as for most viruses, are yet to be elucidated.

B. Large, Enveloped Spheroidal and Elongated Viruses

Numerous, large (70 nm in diameter or greater) animal and plant viruses share the feature of maturing at cell membranes (nuclear, vesicular, cytoplasmic) through which they bud, acquiring an envelope structure in the process. The envelope is composed of both host and viral components, the protein tending to be virus specific, while the lipid and perhaps carbohydrate may be characteristic of the host membrane. Quite often, discernible protuberances called spikes (see Figure 31) are apparent in negatively stained preparations of virions. In cases where they have been most thoroughly studied (ortho- and paramyxoviruses), the spikes are rod-shaped structures about 4–5 × 8–14 nm and appear to be glycoproteins (Compans and Choppin 1973).

Two viruses whose morphologies exemplify numerous spheroidal enveloped viruses are illustrated in Figure 34. They are influenza (a myxovirus) and Rous sarcoma virus (an oncornavirus); some other viruses belonging in these groups are listed in Table 31. Such enveloped viruses tend to be plastic and thus exhibit pleomorphism, which is illustrated in Figure 34 with the influenza virions shown there. While a myxovirus tends to be spheroidal in shape, its nucleoprotein constituent is usually an elongated

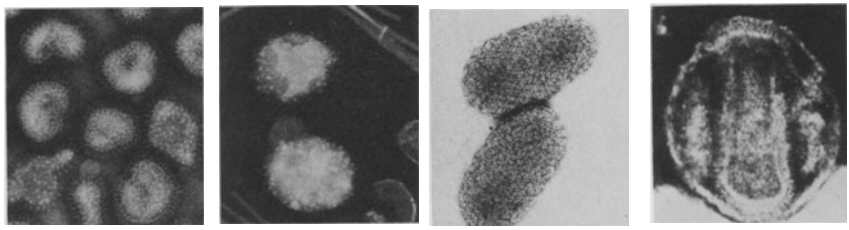

Fig. 34. Three large enveloped viruses and a poxvirus. *a*. influenza virus; *b*. Rous sarcoma virus. *c*. sowthistle yellow vein virus; *d*. vaccinia virus (a poxvirus). Note the pleomorphism and peripheral spikes of the influenza virions. The vaccinia virus particle has been partly stripped with detergent in order to reveal core and lateral bodies. (*a* and *b*, courtesy R. C. Williams and H. W. Fisher; *c*, courtesy D. Peters and *d*, courtesy K. B. Easterbrook.)

structure with helical symmetry that exists in a folded or coiled state within the envelope. A segment of nucleoprotein (nucleocapsid) released from the paramyxovirus, Sendai virus, is illustrated in Figure 34A. This same sort of structure has also been associated with the nucleocapsids of rhabdoviruses.

In both ortho- and paramyxoviruses the nucleocapsid is composed of a single polypeptide species associated with single-stranded RNA in an elongated helical structure similar to that for Sendai nucleocapsid in Figure 34A. However, a basic difference is that the nucleocapsid of paramyxoviruses appears to exist in a single helical structure, whereas that of the orthomyxoviruses may occur in or readily dissociate into several segments (Compans and Choppin 1973). There are hints that such a segmented nucleocapsid may also occur in oncornaviruses (Tooze 1973).

Among the enveloped viruses, herpesviruses are unique in having an icosahedral rather than a helical nucleocapsid. This group of large spheroidal, enveloped viruses has many members, including some with oncogenic properties (see Table 31) (Roizman and Spear 1971).

Another type of large enveloped virus is represented by the group called rhabdoviruses, which has representatives among both animal and plant viruses. Vesicular stomatitis virus, which has bullet-shaped particles (rod with one end rounded and the other planar), is representative of numerous other animal viruses (Table 31) (Hummeler 1971). Such bullet-shaped particles have been observed also with plant viruses, but a bacilliform shape (rod with both ends rounded) seems more characteristic of undegraded particles of these viruses as illustrated by the virion of sowthistle yellow vein virus shown in Figure 34 (see also Knudson 1973). Some other bacilliform viruses in the plant series are listed in Table 34 under potato yellow dwarf, the morphological prototype of this group.

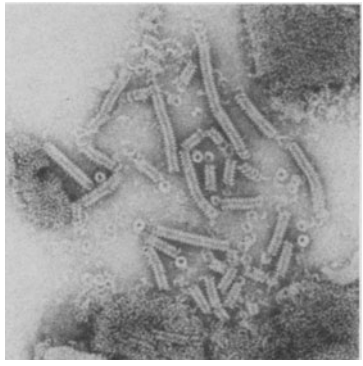

Fig. 34A. A segment of the helical nucleocapsid of Sendai virus. Compare with Fig. 35*c* and 35*d*. (Courtesy R. C. Williams and H. W. Fisher.)

C. Brick-Shaped Viruses

Poxviruses are the largest and most complex of the animal viruses; their virions are usually described as brick- or loaf-shaped. Whether isolated from insects, birds, or mammals (see Tables 31 and 33) (Dales 1973), a basic structural pattern is observed in the virions; they have a highly convoluted, tubular, lipoprotein outer membrane, an internal protein-nucleoprotein core (sometimes called nucleoid), and proteinaceous lateral bodies. These latter features are illustrated by the micrograph of vaccinia virus in Figure 34. In addition to the double-stranded DNA and associated protein, the cores of vaccinia virus enclose four enzymes: a RNA polymerase (transcriptase), a nucleotide phosphohydrolase, and two deoxyribonucleases (DNases)—one an exonuclease and the other an endonuclease. While the function of the lateral bodies is not definitely known, they may serve as inhibitors of the viral DNases since both DNases show elevated activities if the lateral bodies are removed from cores by treatment with a proteolytic enzyme (Dales 1973). Thus the lateral bodies could restrain the activity of the DNases in the vaccinia virions but upon removal during the course of infection might release them to attack host cell DNA.

D. Elongated Viruses

The two basic elongated structures of virions observed thus far are tubular and filamentous particles. They have been noted for several bacterial and plant viruses (Table 32 and 34). Two examples of each type of structure are shown in Figure 35.

Tobacco mosaic virus is the best known and most thoroughly studied rod-shaped virus. The structure of TMV virions was rather well understood by the time negative staining was developed, so this technique only served to confirm the morphology already established by chemical and x-ray studies. Since the approach used for TMV is a classical one for deducing structure of rodlike particles, it will be briefly sketched here.

There was evidence that TMV protein was a single species that occurred in about 2,000 identical subunits (molecular weight about 18,000) per virion of 40 $\times$ 10^6 daltons (Harris and Knight 1955). It was further known that the RNA of TMV was a single-stranded molecule with a molecular weight of about 2 $\times$ 10^6 and about 3,300 nm long, which ran the length of the TMV rod (Hart 1958; Gierer 1957). Important information missing at this time were the arrangement of the protein subunits and the spatial relationship that protein and nucleic acid took with respect to one another. This was supplied by study of the low-angle x-ray scattering patterns yielded by concentrated gels of purified TMV (Watson 1954; Franklin et al. 1957, 1959; Caspar 1956). The x-ray data indicated that the protein subunits of the virus are arranged in a helical array about the long axis of the virus rod; that there is a central hole about 4 nm in diameter so that the rod is actually a tube; that there are regions of high and low density in the particle at specific radii; and, by comparison of radial density distributions of complete and nucleic acid-free particles, that the nucleic acid is not in the center of the tube but is intermeshed with the protein subunits at a radius of about 4 nm. Some of these points are evident from the radial density distribution diagrams shown in Figure 36. As indicated in the figure, density distribution curves similar to that of TMV were also obtained with three strains of this virus and for cucumber virus 4; it will be noted that the curves all show maxima at the same radii and differ mainly in

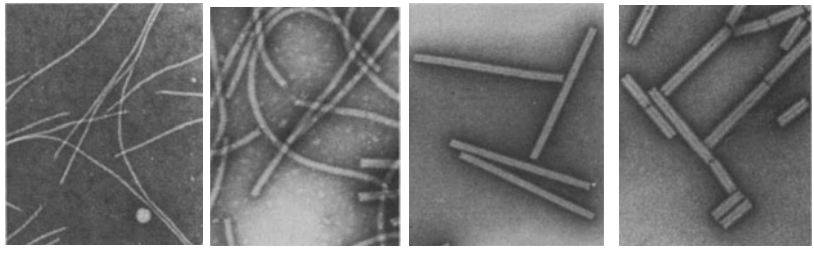

Fig. 35. Some elongated viruses. *a*. coliphage fd; *b*. potato virus X; *c*. tobacco mosaic virus; *d*. tobacco rattle virus. The particles of phage fd and of potato virus X are too long to be shown in their entirety at the magnification used here. (Courtesy R. C. Williams and H. W. Fisher.)

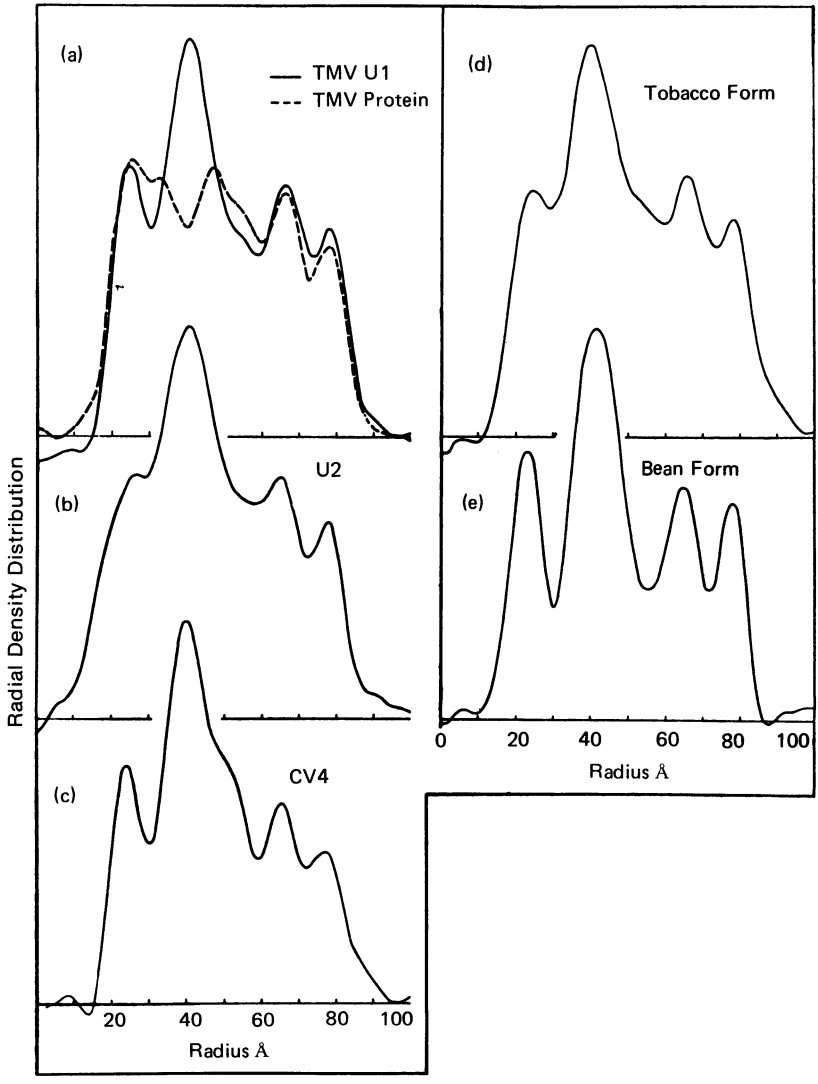

Fig. 36. The cylindrically averaged, radial, electron density distributions of tobacco mosaic virus, some of its strains, cucumber virus 4 (CV4) and TMV protein. The curves show the difference between the electron density of the particles and that of water plotted as a function of radial distance from the particle axis. TMV U1 is common TMV (also called vulgare and wild type) and U2 is a mild strain of TMV (Siegel and Wildman 1954). The strains represented in (*d*) and (*e*) originated in Nigerian cowpea (Bawden 1958). (From Klug and Caspar 1960; see also Caspar 1956; Franklin et al. 1957.)

quantitative respects, which probably represent slight differences in packing of material.

Putting all the evidence together, a model of the TMV particle can be constructed illustrating the helical arrangement of protein subunits in the TMV shell and the manner in which the RNA strand intermeshes with the protein subunits and assumes the helical configuration of the subunits (Figures 7 and 18).

In Figure 35 the central hole is evident in the virions of TMV and of tobacco rattle virus. Cross striations also delineate the helical array of subunits in all of the elongated virions. It will be noted that the filamentous viruses exhibit flexuous shapes rather than the straight form shown by the elongated viruses with greater cross-sectional diameters.

As with other viruses, including the isometric ones, the protein subunits of elongated viruses are associated with the nucleic acid by noncovalent bonds. However, in some cases the stability of this structure is very great; for example TMV has been reported to retain infectivity in extracts at room temperature for 50 years (Silber and Burk 1965).

E. Tailed Viruses

Some bacterial viruses are characterized by spheroidal particles, some by filamentous particles, and many are combinations in which head and tail structures are evident. In the latter case, head capsid may exhibit icosahedral symmetry and the tail helical symmetry. Among the tailless phages there is at least one known, *Pseudomonas* PM2, which has a lipoprotein envelope (Espejo and Canelo 1968; Silbert et al. 1969) that appears to fit closely around an icosahedral capsid. This phage is unusual also in being the only tailless phage possessing double-stranded DNA (which happens to be circular).

The head sizes of different tailed phages vary considerably and the shape ranges from almost spherical to oblong. The head houses the nucleic acid (apparently always double-stranded DNA), while the tail serves as an attachment organ in the initial step of infection and a tube through which the DNA travels in a subsequent step (penetration). Some tails are short, some long, some straight, and some curved; they vary tremendously in complexity, especially with regard to possession or not of accessory structures such as collars, base plates, spikes, tail fibers, and so on. Many of these features are illustrated in Figure 37 and characterize numerous phages, some of which are listed in Table 32.

Tailed viruses have also been observed as infectious agents of blue-green algae (Table 30); two of these are illustrated in Figure 37. The N-1 algal virus (Adolph and Haselkorn 1971), as can be seen in the figure, resembles long-tailed bacteriophages, especially those with contractile sheathed tails. The SM-1 algal virus (MacKenzie and Haselkorn 1972)

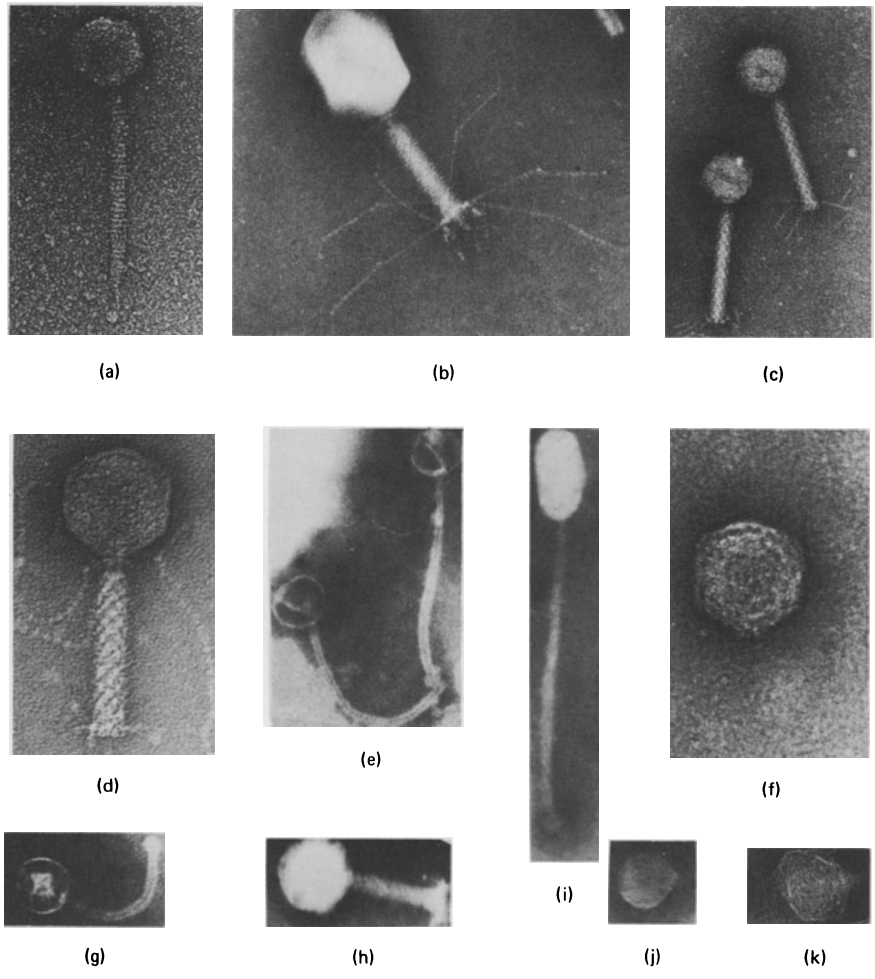

Fig. 37. Some tailed bacteriophages and algal phages. *a*. coliphage lambda; *b*. coliphage T4; *c*. coliphage P2; *d*. N-1 algal virus (from *Nostoc muscorum*); *e*. *staphylococcus* phage 77; *f*. SM-1 algal virus; *g*. *pseudomonas* phage Pc; *h*. typhoid phage Vi 1; *i*. *staphylococcus* phage 6; *j*. a *brucella* phage; *k*. coliphage T7. Mounts were all prepared for electron microscopy by the negative staining technique. (*a, b, c,* and *k,* courtesy R. C. Williams and H. W. Fisher; *d* and *f,* courtesy R. Haselkorn; and the rest, courtesy D. E. Bradley and D. Kay.)

resembles the short-tailed phages; it has an icosahedral head capsid from which there protrudes a collar and a short appendage that could be a tail.

F. Encapsulated Viruses

There are two morphologically different classes of insect viruses that may be called occluded or nonoccluded, depending on whether they typically appear in their mature form in special inclusion bodies or not (Table 33). The nonoccluded virus of *Tipula paludosa* (the crane fly or daddy longlegs), called *Tipula iridescent* virus, is illustrated in a micrograph in Figure 33. However, most insect viruses appear to occur in their mature form in characteristic inclusion bodies. These inclusion bodies are generally crystalline protein packages that contain one or more virus particles. Some of these packages are called polyhedral bodies, and are found characteristically in either nuclei or cytoplasm of infected cells; the diseases associated with them are correspondingly termed nuclear polyhedroses and cytoplasmic polyhedroses. The occluded virions of nuclear polyhedroses are generally rod-shaped, while those of the cytoplasmic polyhedroses are spheroidal and have icosahedral capsids. Hundreds of virus particles are occluded in the crystalline protein matrix of each polyhedral body whether nuclear or cytoplasmic; they can be released by treatment with dilute alkali. For example, the cytoplasmic polyhedrosis virus of the silkworm is released from polyhedra by holding the polyhedral bodies at 25° in 0.1 M NaCl and 0.05 M Na_2CO_3 at pH 10.6 for 1 hr (Lewandowski et al. 1969).

In the insect diseases called granuloses, the inclusion bodies are called granules or, more frequently, capsules. Some distinctions between capsules and polyhedral bodies are

1. Shape of the inclusion bodies: polyhedral bodies occur in a variety of shapes depending on the polyhedrosis involved and have been described as dodecahedral, tetrahedral, rectangular, hexagonal, and crescent-shaped; capsules are usually described as ovoid or egg-shaped in outline although some cubic capsules have been reported.
2. Size: the polyhedral bodies vary in size both in the same and in different polyhedroses but in general they are much larger than capsules and range from 500 to 15,000 nm in diameter, whereas the range of sizes of capsules is more of the order 119 to 350 nm wide and 300–511 nm long.
3. Number of virus particles occluded: hundreds or thousands of virions may be found in polyhedral bodies but on the average only one virion occurs in a capsule.

Thin sections can be made of polyhedral viruses and capsules which upon electron microscopy reveal the dispersion of virus particles (Figure 38). Two concentric membranes can be observed surrounding each virus particle in nuclear polyhedral bodies and capsules but not in cytoplasmic polyhedral bodies. The membranes, when present, are termed inner or intimate membrane (next to the virion) and outer membrane. Their precise functional relationship to the virions is not yet clear. A cross section of a nuclear polyhedral body showing the occluded cabbage looper virus particles and a similar section of a capsule showing a meal moth virus are shown in Figure 38.

As indicated in Table 33 there are at least two other types of occluded insect viruses. One of them occurs in inclusion bodies called spherules and the occluded virus appears to be a poxvirus (Bergoin and Dales 1971); the other is a beetle virus found in peculiar spindle-shaped or ovoid inclusions (Vago and Bergoin 1968).

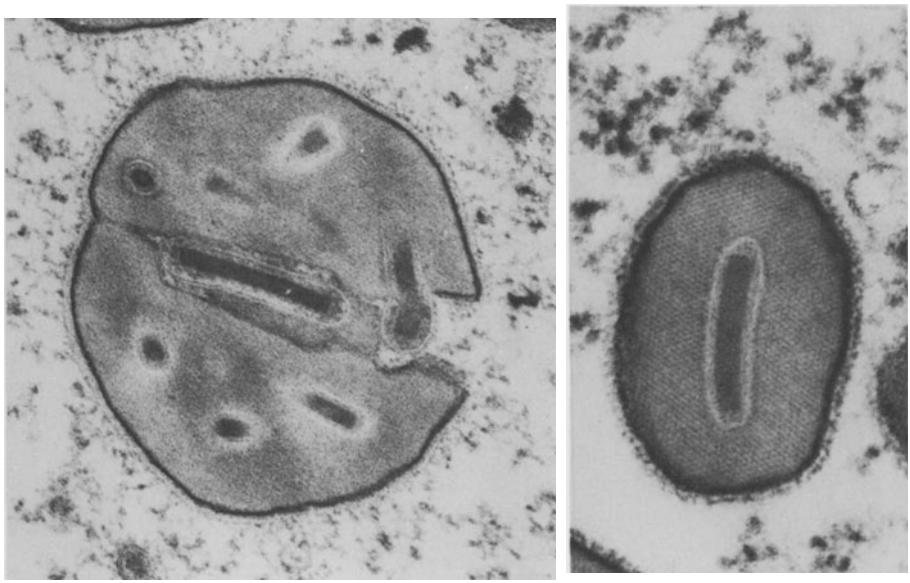

Fig. 38. Two types of occluded insect viruses. *a*. Thin section of a polyhedral body from the nuclear polyhedrosis of the cabbage looper (*Trichoplusia ni*). Bits of the randomly oriented viral rods are apparent in the section with a complete rod discernible in the center of the section. *b*. Thin section from a capsule of the granulosis of the meal moth (*Plodia interpunctella*) showing the single virus particle embedded in a crystalline capsule. (*a*, courtesy M. D. Summers; *b*, courtesy H. J. Arnott and K. M. Smith.)