AN ELECTRON MICROGRAPHIC ATLAS OF VIRUSES

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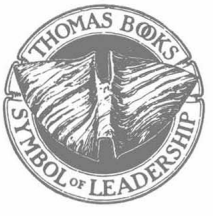

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To Margery and Doris

PREFACE

About three years ago we developed a technique that allowed virus particles to be photographed in the electron microscope without subjecting them to the heavy electron irradiation which they undergo during traditional electron microscopy. The first results, obtained on bacteriophage T4 and tobacco mosaic virus, showed that the method allowed discernment of details of structure that were finer than any previously shown. We then began to examine other viruses available from our colleagues in the Virus Laboratory, and fairly soon we had a respectable collection of electron micrographs. It occurred to us that others interested in the structure of viruses might wish to see our photographs, particularly if we could enlarge the collection to include examples of viruses of many known structural types. Thanks to the generosity of colleagues in many places, who gave us samples of viruses which we did not have, we have been able to assemble an *Atlas* containing electron micrographs of some thirty-one different viruses.

The *Atlas* is intended for two classes of people: those who are investigators in virus research and who would find it useful, in either their research or teaching, to have available a fairly extensive collection of electron micrographs; and those whose interest in viruses is secondary, or just beginning, but who would like to learn more about their visual appearance. The brief accounts accompanying the Plates in the *Atlas* are intended for the latter audience, since they are not intended to be extensive nor highly technical. We hope that, in examining the micrographs in this *Atlas* the reader will be struck, as we are, by the fantastically diverse ways in which Nature has assembled large molecules into ordered structures.

All of the electron micrographs in the *Atlas* were obtained by one or the other of us in the senior author's laboratory, and all were obtained by use of the technique of minimal electron beam exposure (Williams, R. C., and Fisher, H. W., *J Mol Biol, 52:* 121, 1970). Most of them were photographed at the initial magnification of × 40,000, either in a Siemens 1A or a JEOL 100B electron microscope, both equipped with anti-contamination devices. The support films for specimen deposition were carbon over collodion; negative staining was almost always by use of sodium phosphotungstate. Photographic enlargements were on Eastman bromide paper, grades F-3 to F-5. The magnifications at which the micrographs are presented are not uniform; rather, each picture is at a magnification only great enough to show the smallest detail of structure which is unequivocally present.

The arrangement of the thirty-one micrographs in the *Atlas* is based upon the considerations discussed in the Introduction and shown in its accompanying chart. At the beginning of the account accompanying each Plate is a very brief description of the chemical and physical properties of the virus: the type and strandedness of the nucleic acid and its percentage content, the shape of the virus particle and whether it is naked or enveloped, and its dimensions.

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INTRODUCTION

Background

Ever since the discovery in 1935 that the virus of the mosaic disease of tobacco was a chemically defined macromolecule, rather than a mysterious living substance, the elucidation of virus structure has been the subject of much study. For several years, however, the small size of viruses precluded attempts at their direct visualization by optical means, and limited the structural investigations to determinations of their sizes and shapes by physical methods such as ultrafiltration, sedimentation and diffusion analysis, and viscometry. Only the pox viruses were large enough to be seen by bright-field, light microscopy. Dark-field microscopy was capable of revealing objects believed to be the particles of the smaller viruses, but such imagery could establish only imprecise estimates of shapes and sizes. At the close of the 1930's our knowledge of virus structure was limited to the realization that several viruses were approximately isometric, with diameters covering a range from 15 nm to 150 nm, and that one, tobacco mosaic virus, was rod-shaped with a length, approximately 300 nm, about twenty times its diameter. Nothing was known about any finer scale structure. With the advent of the electron microscope the exploration of the physical properties of viruses changed dramatically. In 1939 the first electron micrographs of viruses were obtained, showing that, indeed, tobacco mosaic virus was a rod, that other viruses showed images interpretable as arising from spheres, and that still other viruses looked like tadpoles. While these first pictures were not even second-class by present standards, they did afford exciting promise that the electron microscope would eventually be a powerful instrument in delineating virus structure.

The word *structure* as applied to objects the size of viruses may mean different things to different investigators. An analytical chemist may say that he knows the structure of a protein molecule if he has determined the sequence of its amino acid residues. The physical biochemist may be primarily interested in size, shape, density, and water of hydration of such a molecule, with its distribution of electric charge included for good measure. An x-ray analyst wants the three-dimensional localization of the atomic scattering centers within the molecule when it is part of a crystalline assembly, but must remain content to be relatively ignorant, from his own work, of the chemical identity of the scattering centers. The electron microscopist seeks information similar to that found by the x-ray analyst. His methods have certain advantages and disadvantages. He starts with the supposition that a virus particle does not consist of material which has a uniform density over all the volume occupied by the particle; that there are regions from which water is highly excluded and regions consisting

mostly of water, and that even within the former regions the density may vary because of differences of chemical composition. These local differences in density constitute his *structure* of the particle. The actual specimen examined is a single particle, hopefully treated to accentuate the differences in water content and chemical composition, and it must be photographed after it has dried from its normal environment. Since all the optical information comes from single particles the problem of distinguishing signal from noise is severe, and since the photographs are two-dimensional projections of three-dimensional objects oriented at random the problems of interpretation are not simple.

While in principle the electron microscope will resolve details in the near-atomic size range, as does x-ray analysis, in practice the resolution limit in electron micrographs of viruses is not nearly so fine. The x-ray analyst uses a crystal as a specimen; consequently he is presented with the redundancy of information arising from a periodic arrangement of structure and is able to enhance notably the signal-to-noise ratio. By use of computational methods the structural information can be presented in three-dimensional form at a high level of resolving power. But x-ray analysis of this nature is restricted to objects that will crystallize and are relatively simple in structure, and it is laborious and time-consuming. On the other hand, electron microscopy will yield some information about any object within its workable size range, whether or not the particle is crystallizable, and the application of its methods, while requiring skill and judgment, does not take much time. The methods of x-ray analysis and electron microscopy are complementary rather than competitive.

Specimen Preparation

An electron micrograph of a particle like a virus that has simply been allowed to dry out of its aqueous environment is unimpressive. The contrast in the image is low, evidence of inner structure is not usually present, and the object appears obviously flattened by the forces of surface tension during drying. Even though the electron microscope has ample resolving power to show fine detail, there is no evidence of that capability in the micrograph. Almost all the improvements in the practical micrography of purified virus particles have been directed toward the problems of contrast and preservation of three-dimensional structure of the specimen. Contrast in the image of an untreated virus particle is poor because the electrons passing through different portions of it are all scattered to about the same degree; i.e. in a dried particle of organic material every electron traverses about the same mass of material. Contrast would be improved if certain regions of the particle, related to its external or internal structure, could be stained with a substance of high density (high electron scattering power). The first staining of this nature, a surface *stain,* was accomplished by the shadowing technique, introduced in 1944. A thin film of a heavy metal, such as uranium, was vacuum-deposited at an oblique angle upon the dried virus particles, thus creating variations in the thickness of the film wherever the particle surface had humps and hollows. Regions of thicker or thinner metal film introduced more or less electron scattering, resulting in greatly enhanced contrast based upon surface topology. While

the results were striking, and while the technique had universal application for about fifteen years, it always suffered from three defects: It did nothing to alleviate the artifacts of drying, it obscured whatever inner structure might be present, and it produced a fine-scale granulation on the particle surface because of the crystallite structure of the metal film.

In the early 1950's two successful methods were developed for preventing the artifacts that come about when virus particles dry out of water. In one, the *critical point* method, the specimen is transferred from a water solution to a solvent that is miscible in both water and liquid CO_2, at high pressure and room temperature. The pressure is then reduced and the specimen dries from a gaseous environment; thus, no water-air interface passes over it. The other method was simply an application of freeze-drying to electron microscopy, wherein the specimen is dried out of an ice matrix upon vacuum sublimation at low temperature.

During most of the years in which the shadowing method was extensively used for revealing the surface structure of virus particles little serious attention was given to the development of chemical stains for this purpose. It was recognized that the large gamut of stains available to light microscopy would not be applicable to electron microscopy, since these stains act by the selective absorption of certain regions of the visible spectrum, thereby providing differentiation of stained structures by their differences of color. The only staining effect that can be observed in the electron microscope is one that is brought about by differences of mass per unit area of the specimen that is traversed by the electrons. This fact requires that good electron stains be compounds of heavy metals such as tungsten and uranium, if the volume occupied by the stain is to be kept at a desirable minimum. With this restriction it could not be expected that stains with a high degree of chemical specificity could be found, as distinct from the situation in light microscopy.

In 1959 a highly successful technique was developed for enhancing the contrast in images of particles, such as viruses, by the use of heavy metal salts. The method was called *negative staining,* although a more apt description is *heavy metal embedding.* The process is the essence of simplicity: An aqueous suspension of virus particles is mixed in equal volume with a 1 to 4 percent solution of a heavy metal salt, such as sodium phosphotungstate (PTA), and the mixture allowed to dry on the electron microscope specimen film. The dried specimen consists of virus particles embedded in the residue of the stain that remains after drying. Wherever the particle has a hollow region, or a surface indentation, initially filled with water, it will now contain a mass of stain. Wherever there is a protuberance there will be less stain. Electrons passing through the hollow or indented regions will experience more scattering, because of the local mass of dried stain, than will electrons traversing a region where there is a surface protuberance. Thus, the electron image will have areas that are relatively dark and light, corresponding to regions in the particle that are relatively thin and thick, as seen by the traversing electrons. The contrast is thus inverse; thick specimen areas appear electron lucent and thin areas appear relatively electron opaque. This effect was the origin of the term *negative stain.* Since a very thin film of a dense material such as

PTA will create a notable enhancement of electron scattering, the power of this method to delineate very small structural differences is great.

An unanticipated, but enormous, benefit of the method of negative staining lies in its relatively good preservation of three-dimensional structure during drying of a virus particle. As water is removed the space occupied by it is filled with a matrix of stain which becomes progressively harder as drying proceeds. Thus, the particle cannot greatly flatten and distort during preparation for electron microscopy. It is safe to say that negative staining has gone far to solve two problems of specimen preparation at one blow: introduction of fine-scale contrast in the electron image, and preservation of structure during drying.

The virtue of negative staining, that it discloses structure in any part of a particle accessible to the stain, also produces a disadvantage in the interpretation of micrographs. Suppose a virus particle has protuberances all over its surface. The stain will enter the hollows between the protuberances regardless of whether they lie on the part of the virus surface that is *below* (next to the specimen film) or *above* (farthest from the film). In addition, some stain may enter the interior of the particle. Since the electron image is a two-dimensional projection of all the structural details on and within the particle, as revealed by the negative stain, its interpretation may be far from straightforward. Recently, stereoscopic micrographs (obtained by tilting the specimen between two successive photographic exposures) have been used to help unscramble the puzzle of overlapping regions of contrast.

The general method of negative staining described above occasionally requires modification in actual practice, and it may not always produce ideal results. Each stain used has a limited pH range, and the virus sample may suffer some structural degradation at pH's within this range. Some viruses are not stable in pure water and must be kept in an ionic solution. If the necessary solute is fairly concentrated and is nonvolatile, and is present when the negative stain is added, it may create a coarse granulation in the dried negative stain. A solution to this dilemma is to dialyze the virus sample into a volatile buffer, such as ammonium acetate or ammonium bicarbonate, at an appropriate pH and ionic strength. Another problem has to do with uneven spreading of the virus-containing negative stain upon the specimen film. If the virus concentration is low ($\approx < 10^{12}$ particles/ml), the stain tends to dry in patches that are either too thick or too thin to be useful. One way to minimize this problem is to add material that will aid uniform spreading. Some of the Plates in this *Atlas* (such as Plate VIII) show particles of potato virus X as well as the virus of interest; the long, sinuous particles of potato virus X were added to help create a uniform film of dried stain. More recently it has been found that if the carbon specimen-support film is treated to a high-voltage glow discharge in a partial vacuum it will be rendered quite hydrophilic and uniform staining will result. Many of the following Plates are micrographs obtained after treatment of the support films in this manner.

One last problem in specimen preparation is the tendency of the particles of some viruses to flatten and partially disintegrate upon even the gentlest drying in negative stain. The herpes simplex virus (Plate XXVI)

is an example of this extreme fragility. The disintegration of virus particles during drying can be informative since sometimes internal structures are best revealed after partial disruption (Plate V, Sendai virus).

It has been recognized only recently that the procedures involved in the actual electron micrography of a particle as small as a virus may be quite damaging to its finest details of structure. Traditional electron microscopy involves using the actual virus particles which are to be photographed as the reference objects during focusing of the electron image on the viewing screen. The attendant exposure of the particles to a fairly intense beam apparently volatilizes some of the material of the virus and even rearranges the distribution of negative stain. Fortunately, it is possible to minimize the damaging effect of the electron beam by use of a portion of the specimen near the region of interest for performing the operations of focusing, leaving the important region unirradiated until the instant the photograph is taken. This technique has been called *minimal beam exposure;* it has been used in obtaining all the electron micrographs in this *Atlas.*

Principles of Virus Structure

Prior to about 1955 there was little thought given to whether viruses in general had any regularity of structure, any evidence that they were built on architectural plans. To be sure, x-ray analysis of tobacco mosaic virus had strongly intimated that its structure was that of a helix, electron micrographs of some small, freeze-dried viruses showed them to have an hexagonal outline, and the surface of rabbit papilloma virus was known to exhibit protuberances in a regular array. But so far as most viruses were concerned, they were known by the chemist to consist of protein and nucleic acid, with occasionally some lipid, and were recognized by the electron microscopist only to be particles which were frequently uniform in size and were shaped like bricks, spheres, rods, or tadpoles.

With the acceptance of the notion of RNA-protein translational coding a new way of looking at virus structure was inevitable. The genomes of many viruses clearly contained so little coding capacity that the proteins of their coats could not be made up of molecules larger than about 50,000 daltons. Some viral proteins had been analyzed and found to be in this range of molecular weight. Since the total amount of protein in the coat of even a small virus was at least 3 to 4 $\times$ 10^6 daltons, it became evident that the entire coat protein must consist of multiple copies of smaller units. Furthermore, x-ray analysis of crystals of two small, spherical viruses had shown that the particles were built with exquisite symmetry, an indication of a highly organized physical structure. Shortly after the introduction of negative staining there were increasing reports of regularly arrayed structures on the surfaces of virus particles. The time was ripe to devise a model system of construction, a set of architectural principles, hopefully for all viruses.

A puzzling aspect of the early micrographs showing regular surface structures on virus particles was that, in those cases where the number of protuberances could be calculated from the micrographs, it always ended

in the number *2;* e.g., 12, 32, 42···252··. Various schemes were postulated to account for this numerology, but in 1962 Caspar and Klug uncovered the principles upon which the protein coats of virus particles, at least the simpler ones, are built, and from these principles the magic number *2* lost its element of mystery. They started with the observation that the simpler viruses, except for the tailed bacteriophage, are either rod-shaped or isometric. In the former case, the principle of construction is simple: The particles are built with helical symmetry, since this is an arrangement that would impart minimal energy to an aggregate of identical protein molecules bound in strictly equivalent positions to other such molecules and to the nucleic acid polymer. For isometric particles the minimal energy state is met if all the protein subunits are in equivalent positions; i.e. each subunit is bound to its neighbors exactly like every other subunit. It could be shown that an isometric form would result only when there were 60n such subunits; it would then have the symmetry elements of an icosahedron. (Icosahedral symmetry is also known as 5 3 2 symmetry, meaning that axes of 5-fold, 3-fold, and 2-fold symmetry may be passed through the center of the particle.) Such symmetry seemed reasonable as a principle of construction, since the earlier x-ray work had shown intimations of 5 3 2 symmetry in small virus particles.

At this stage the theory of Caspar and Klug would not explain the mysterious number *2,* nor why some viruses like adenovirus (Plate XXIV) showed angular contours and planar facets. An isometric shell built with 60n subunits in equivalent bonding positions would be expected to exhibit a circular contour. This dilemma led to the concept of quasi-equivalence: bonding which is almost identical for every subunit. With only small energy increments due to strain distortion an isometric object can be built of subunits, having icosahedral symmetry and having the observed planar facets; in the extreme the object would actually be an icosahedron, like adenovirus. Next came the notion of clustering of the protein subunits. There is no energetic reason why groups of subunits could not cluster; the only problem was to devise a clustering that would be in accord with the electron microscope results (12, 32··252 visible protuberances). It could be shown that clustering of the subunits into dimers, trimers, pentamers, and hexamers would satisfy the symmetry requirements. It actually turns out that pentamer-hexamer clustering is by far the most common, only tomato bushy stunt virus (Plate XII) having been found to have a different clustering, the dimer.

The regular array of protein, or structural, subunits forming the closed shell of a virus particle is called its *capsid,* and, as noted above, the array may have either helical or icosahedral symmetry. The structure that is composed of the capsid and its enclosed nucleic acid is called the *nucleocapsid.* The simplest capsid of an isometric virus would have 60 structural subunits. These would cluster around the 12 vertices of the equivalent icosahedron. Such a particle (the *virion* of the virus) would appear to have 12 visible protuberances, or morphological units, or *capsomers.* Elaborations beyond this primitive arrangement are most readily understood if it is imagined that the structural units are arrayed on the surface

of a polyhedron that is either an icosahedron or a pentagonal dodecahedron. (They may not be actually arrayed on such a surface, but their symmetry would still be 5 3 2.)

An icosahedron is a polyhedron built of 20 identical, equilateral triangles, having 12 vertices and 30 edges. The dodecahedron is a polyhedron built of 12 identical pentagons, having 20 vertices and 30 edges. While virions are known that are built on the dodecahedron as the base, the elaboration of capsomeric structure is easiest seen with the icosahedron. The 12-capsomer virion may be thought of as having three identically spaced protein subunits per triangle of the icosahedron; 60 subunits in all, clustered as pentamers near the 12 vertices. But each of the 20 triangular facets of an icosahedron can be divided into equilateral sub-triangles whose number, T, is given by $T = Pf^2$, where $P = 1$ and $f = 1, 2, 3 \cdots$. In the case of the icosahedron, T (the *triangulation number*) is thus 1, 4, $9 \cdots$. Each sub-triangle must contain three subunits in identical, equal-spaced array. The entire capsid of a $T = 4$ capsid would then contain 240 subunits $(20 \times 3 \times 4)$. Those nearest the 12 vertices will cluster into pentamers. The remaining 180 (240 *minus* 60) subunits can cluster into a regular array of hexamers, giving the entire capsid a surface studded with $12 + 180/6 = 42$ capsomers in regular array. When $T = 9$ the number of capsomers will be 92; $T = 16$ gives 162, etc. Thus, with pentamer and hexamer clustering, the number of units observable in the electron microscope, the capsomers, will always end in *2,* since the number of pentamer clusters will be 12 and the number of hexamers will be a multiple of 10. Figures 1 and 2 show models of an icosahedron upon which structural subunits and capsomers have been drawn for the cases of $T = 1$ and $T = 3$.

If the basic polyhedron is the pentagonal dodecahedron the equation $T = Pf^2$ still applies, but now $P = 3$. Hence T takes on the values 3, 12, $27 \cdots$, and if the subunit clustering is pentamer-hexamer the number of observable capsomers will be 32, 122, $272 \cdots$.

Capsid arrangements falling into neither the $P = 1$ or $P = 3$ class may exist. Closed structural shells with 5 3 2 symmetry, in which the

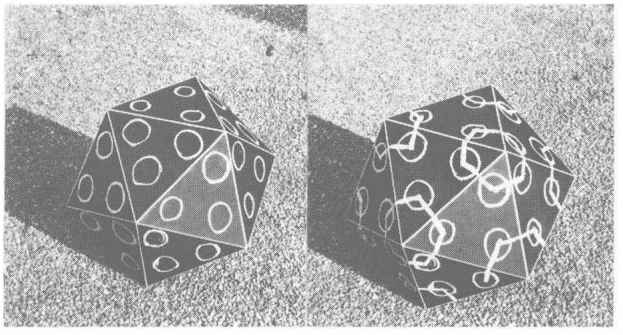

Figure 1. Model of an icosahedron upon which has been sketched circles to show structural subunits of the *capsid,* and connected circles to show clustering of the subunits. In this, the $T = 1$ arrangement, the 60 subunits cluster in pentagonal array at the icosahedral vertices.

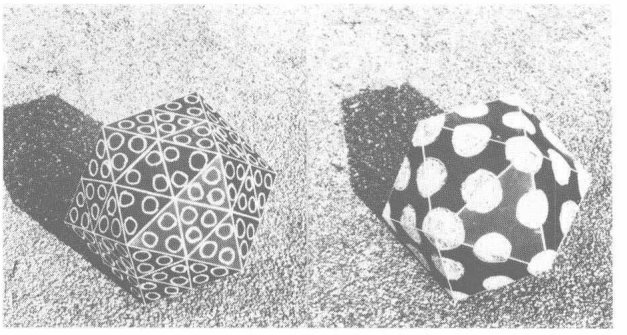

Figure 2. Same as Figure 1, except that the $T = 4$ arrangement is illustrated. Spots drawn on the model show location of structural subunits in hexagonal clustering with pentagonal clustering at the vertices.

subunit bonding is quasi-equivalent, can be constructed with any value of T that is given by the relation $T = Pf^2$. While the two smallest values of P are 1 and 3, as mentioned above, the general expression for P is $P = h^2 + hk + k^2$, where h and k are integers with no common factor. ($P = 1$ when either h or k is zero; $P = 3$ when $h = k = 1$.) Only one class of virion has been found, however, with $P > 3$: Virions of the Shope papilloma (Plate XXI) and the human wart viruses have been found to be constructed according to $P = 7$, $T = 7$. For all T-values, however, if the subunit clustering is pentamer-hexamer, the following two relations hold:

S = number of structural subunits = $60\ T$

M = number of capsomers = $10\ (T - 1)$ hexamers + 12 pentamers.

The various numbers of capsomers that can appear on virons of any class would be: 12,32,42,72,92,122,132,162, $\cdots 252 \cdots$. Many of these numbers have been reasonably well established as actually existing in the capsid structure of one or more viruses. Whether trimer clustering of subunits exists in any viral capsid, and whether the dimer clustering found in the capsid of tomato bushy stunt virus is the only example of its kind, remain to be seen.

A determination of the number of capsomers on the surface of virions of a particular virus may not be a simple matter, for at least three reasons. The virus particles may be distorted upon drying in the negative stain, thereby suffering displacement of the capsomers from their normal positions. Secondly, the capsomers on both the "top" and "bottom" surfaces of the virion will usually be projected upon the electron micrograph and may be out of register. Thirdly, the capsomers on the entire virion cannot actually be counted, one by one, since they cannot all be discerned. Capsomer *counting*, instead, comes from a simple calculation based upon observations of capsomer *arrangement*. Under the most favorable circumstance, where many particles in the electron micrograph show "one-sided" contrast and where some are appropriately oriented, it is possible to identify capsomers that are surrounded by six nearest neighbors *(six-coordinated)* and some that are surrounded by five *(five-coordinated)*. The latter will be at the vertices of the icosahedral array. If the orientation of some of the virions is such that two or more five-coordinated capsomers can be found it is usually possible to detect the number and arrangement of the six-coordinated ones lying between any two five-coordinated ones. This information will allow the T-number to be calculated. In the case of virions in the $P = 1$ class, the numerology is simple. If the number of intervening six-coordinated capsomers is zero, then $T = 1$; if the number is 1,2,3,4 $\cdot\cdot$ the T-numbers are 4, 9, 16, 25 $\cdot\cdot$. The clearest example of the application of this kind of examination is seen in Plate XXIV, adenovirus. Some of the virions show a triangular facet. The capsomers at the vertices are five-coordinated, with four six-coordinated ones intervening. Thus, the capsid of adenovirus has a $T = 25$ icosahedral lattice, with a total of 252 capsomers and 1,500 structural (protein) subunits. The $P = 3$ class leads to more complicated capsomeric patterns, except for the primitive $T = 3$ case. Here, any two five-coordinated capsomers

will be joined by two six-coordinated ones in a manner such as to produce a diamond pattern, with the latter capsomers at the obtuse angles. In a particularly favorable orientation (a view down a 2-fold axis) a diamond pattern on the top surface will be in register with one on the bottom surface, yielding a pattern that is quite distinctive. Such "diamonds" can be seen on some of the virions of turnip yellow mosaic virus (Plate VIII).

The classical architectural scheme for nonenveloped, isometric viruses refers only to their capsids; nothing is said about the structural arrangement taken by the nucleic acid. From several lines of evidence, notably resistance of the nucleic acid of intact virions to nucleolytic attack, the nucleic acid is universally believed to be in the interior. But there is no firm evidence as to whether it is arrayed in a fashion that accords with the symmetry of the virion capsid. Only for tobacco mosaic virus is the form taken by the RNA known: a helical path, buried within the capsid and having the same pitch as the protein portion of the helix.

Some of the more complex viruses contain their nucleocapsids within an outer envelope. The nucleocapsid may have either icosahedral symmetry (Plate XXVI, herpes simplex virus) or helical symmetry (Plate V, Sendai virus). The latter have their nucleic acid bound to protein subunits in a helical array very much like that of tobacco mosaic virus. The nucleocapsids, or cores, of the complex virus are usually not seen in negatively stained, intact virions, although such structure can be discerned in reovirus (Plate XIII). The most striking display of nucleocapsid material is found in the disrupted virions of Sendai virus.

Virus Nomenclature and Classification

Both the naming and the grouping of viruses have been notoriously controversial subjects for many years. Historically, the rational development of nomenclature and taxonomy of viruses has been hindered by the fact that investigators from a wide variety of disciplines have discovered or isolated new viruses and have had differing opinions about the importance of the various viruses and their disease characteristics. Investigators have included biologists, physicians, entomologists, veterinarians, pathologists, and even molecular biologists, among others, so it should be no surprise that the only common attribute of the popular names ascribed has been the terminal word *virus*. For example, this *Atlas* includes virus names which describe the appearance of the infected host (tomato bushy stunt virus, tobacco mosaic virus), the town or village of isolation (Sindbis virus, Sendai virus), the diseased tissue (poliomyelitis virus, vesicular stomatitis virus), the discoverer's name (Rous sarcoma virus), acronyms (reovirus), and even coded symbols from laboratory notebooks (bacteriophage T4 and ØX174). However, since little confusion seems to have come from the use of the popular names, they have been used in this *Atlas,* rather than some Latin binomials or other system of nomenclature that has been proposed. To paraphrase Gertrude Stein, to an electron microscopist "a virus is a virus is a virus."

The customary natural grouping of the viruses by early workers was rather operational and provincial. Those who worked with plants were concerned only with the classification of plant viruses and plant virus

diseases; similarly for those working with animal, bacterial, and insect viruses. Even much later, those international commissions and provisional committees formed to make recommendations for codes and classifications have been restricted to consideration of general groups of the virus hosts. For example, in 1966 the Plant Virus Subcommittee of the International Committee on Nomenclature of Viruses was established to make nomenclatural recommendations on viruses infecting plants. In 1971 they reported on sixteen groups of plant viruses. Some members of the Subcommittee, however, did not share the views of the others, and the suggestions have continued to be unofficial and must endure the test of time. It is not clear that the viruses themselves recognize and adhere to some of the proposed classifications. For example, bacteriophage seem not to recognize the taxonomic boundaries in microbiology, and some so-called plant and animal viruses multiply in insect hosts as well as in their recognized plant or animal hosts. *In vitro* systems of subcellular compositions have been devised which cross taxonomic boundaries for the growth of some bacteriophage components in animal cell systems and for the growth of some animal virus nucleic acid in purified enzyme systems from *E. coli*.

Even though continuation, for the present, of the use of popular names of viruses seems justified, it is difficult to arrive at a sequence for the presentation of the pictures of viruses in this *Atlas* without entering into "the religious war of taxonomy." A collection of electron micrographs of viruses portrays only what one sees, and so its arrangement is logically governed by the properties of structure, the molecular biology, and the geometry of the capsids of the virions. Since viruses can be distinguished from the rest of the biological world because they contain either RNA or DNA, but not both, a grand subdivision based on the chemical nature of their genetic material is universally accepted. Lwoff, Horne, and Tournier proposed a system of virus classification (the LHT system) in which a division on the basis of the kind of nucleic acid was the first of four discriminating characteristics or "essential integrants." The remaining three relate to what is seen in a high-resolution electron micrograph of a virion. The four characteristics are:

1. The chemical nature of the genetic material, either DNA or RNA, determining the subphyla.
2. The symmetry of the nucleocapsid of the virion: helical, cubic, or binal, determining the classes.
3. The covering of the nucleocapsid, either naked or enveloped, determining the orders.
4. The size of the nucleocapsid, either the diameter of the helix or the number of capsomers in the cubic system, determining the families.

The way these criteria have been utilized in the *Atlas* is shown in the accompanying chart that lists the popular names of typical family members to be found in the *Atlas*. Attention should be called to some special features of organization: (1) the helical arrangement of the nucleocapsid of the bullet-shaped vesicular stomatitis virus is very different from that of the influenza and Sendai viruses which are also helical RNA viruses

with envelopes; (2) Rous sarcoma virus does not yet have its nucleocapsid structure established; and (3) the binal DNA viruses (i.e. the phage with isometric heads and helical tails) have been separated according to whether they are virulent or temperate. Although the chart shows subdivision of orders into families only on the basis of relative size, the LHT system specified the exact number of capsomers and triangulation number for viruses with cubic symmetry, and the diameter for viruses with helical symmetry. Details about these structural characteristics can be found in the discussion which accompanies each Plate. Lwoff and Tournier have further suggested that the families may be subdivided into many genera according to additional characters such as the number of strands of nucleic acid and its circularity, base composition and nucleotide sequence, the molecular weight of the structural proteins, immunochemistry of the capsomers, enzymes determined by the genetic material, site of synthesis of viral materials, virus-cell interactions, host specificity, virulence and symptomology, to mention a few. These fine distinctions are clearly beyond the scope of the organization plan of the *Atlas*. Many of these properties are mentioned in the discussion accompanying each Plate.

Suggestions have been made that a cryptogram should follow the vernacular name of a virus in order to describe in shorthand fashion a few salient facts about the virus. Such a cryptogram would contain four pairs of symbols with the following meanings: the first pair of symbols to state the type of nucleic acid and its strandedness; the second pair to give the molecular weight of the nucleic acid and its percentage content in the virion in the particles; the third pair to specify the outline of the shape of the entire particle and the outline of its nucleocapsid; and the fourth pair to name the kinds of hosts and kinds of vectors. Since the information added by a cryptogram depends upon the reader's memory of the meaning of the symbols, cryptograms have not been included in the *Atlas*.