Archiv für die gesamte Virusforschung 32, 66—72 (1970)

Some Implications of a Morphologically Oriented Classification of Viruses

By

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With 1 Figure

Received March 2, 1970

During the ten years that have elapsed since the electron microscope technique of negative staining was applied to virus particles (BRENNER and HORNE, 1959), it has become clear that the morphology of a virus is an integral and essential aspect of our knowledge about it. For example, two diseases of suspected viral aetiology, progressive multifocal leucoencephalopathy (RICHARDSON, 1961; ZU RHEIN, 1967) and subacute sclerosing panencephalitis (DAYAN, 1969), now have particular viruses associated with them as the result of electron microscopy (WATERSON and ALMEIDA, 1969). In addition, a group of viruses recognised biologically, but otherwise uncharacterised, have now been linked with others, unsuspected of affinity with them, merely on the basis of their distinctive morphology. In fact the name given to this group (coronaviruses) is derived from the distinctive fringed appearance of the particles as seen in the electron microscope (ALMEIDA and TYRRELL, 1967).

It is now accepted without question that groupings of viruses are acceptable only if they place within a single group viruses which are morphologically identical (LWOFF *et al.*, 1962; ALMEIDA, 1963; ANDREWES, 1970). It is an admission of our limited knowledge of the arboviruses that this group still contains a morphologically heterogeneous collection of viruses, and it seems likely that, although the term arbovirus will continue to be significant biologically, it will be used progressively less as a fundamental scientific grouping. Many of the now well established relationships among viruses were suspected only when the morphology of a particular virus became known. For example the coronaviruses (ALMEIDA *et al.*, 1968a) mentioned above contain the unlikely mixture, when considered from the point of view of disease, of some human common cold viruses, infectious bronchitis of fowls, mouse hepatitis virus, and a gastro-enteritis virus of pigs (TAJIMA, 1970; THROWER and BRUCE, 1970). Again the herpesviruses range, as agents of disease, from equine abortion to pseudo-rabies, with some interesting members in between. No example has yet been found of a virus

which exhibits the appearance of an established group but does not have the biochemical and biophysical properties of the group, so that, for example, all viruses with the appearance of adenoviruses have DNA, are ether-resistant and are formed in the nucleus of the cell. On this basis it seemed worthwhile to re-examine in the light of present knowledge the pattern obtained when morphology is the prime criterion for a classification of viruses.

The proposed arrangement is set out in Fig. 1. It follows the previous convention of an initial division into RNA and DNA viruses. The next division is into the two types of symmetry associated with viruses, *i.e.* cubic and helical. A new departure here is a third category "no obvious symmetry". As will be seen later, this is in fact not a grouping of incompletely characterized viruses, but a positive categorization with biological significance. A third division is made horizontally, across the nucleic acid type, into viruses that are ether-stable and those that are ether-labile. Within these groupings, the arrangement is further influenced by the molecular weight of the viral nucleic acid. At the left of the figure a brief description of the composition of each grouping has been added. The groups illustrated are by no means exhaustive, *e.g.* bacterial viruses have been included only where they supplied a type of symmetry not to be found among animal viruses. Where a morphological class can be designated by a group name, *e.g.* poxviruses, adenoviruses, coronaviruses, this has been used. Otherwise, well known members of a morphological group have been used as type viruses, for example, "parainfluenza-measles" (ANDREWES and PEREIRA, 1967). In this context, it should also be remarked that, although a single micrograph was used to illustrate the group, parainfluenza and measles are type members of two biologically distinct virus groups (WATERSON and ALMEIDA, 1966). Some of the viruses used as type members may be unfamiliar, and references are given here for them. (Cat respiratory virus; ALMEIDA *et al.*, 1968a: African swine fever; ALMEIDA *et al.*, 1967: Marburg agent; ALMEIDA *et al.*, 1970: Adenovirus satellite; MAYOR *et al.*, 1965).

The most interesting division, and perhaps the most significant in relating biological properties to structure, is that produced by the line dividing the ether-labile from the ether-stable viruses (ANDREWES and HORSTMANN, 1949). Those viruses above what might be described as the ether line are of the simple type, *i.e.* they have an arrangement of nucleic acid and protein which displays obvious symmetry. Each one of the viruses in this category would, if obtained in sufficient purity, be expected to yield proof of their essential symmetry when examined by X-ray diffraction by revealing patterns referable either to cubic or to helical symmetry. However, when we consider the viruses below the ether line the pattern is a very different one. The main reason that they are sensitive to organic solvents, such as ether, is the possession of a cell-derived and therefore lipid-containing outer envelope. In the intact state few of these viruses give any hint of symmetry, and it is only when disrupted that symmetrical components may become accessible to electron microscopy. Even if these viruses could be obtained in a highly purified form there would be no question of them crystallising like the simpler forms above the ether line.

If we now add the parameter of nucleic acid content to this consideration of virus symmetry we see that it ranges at one extreme from the defective (satellite)

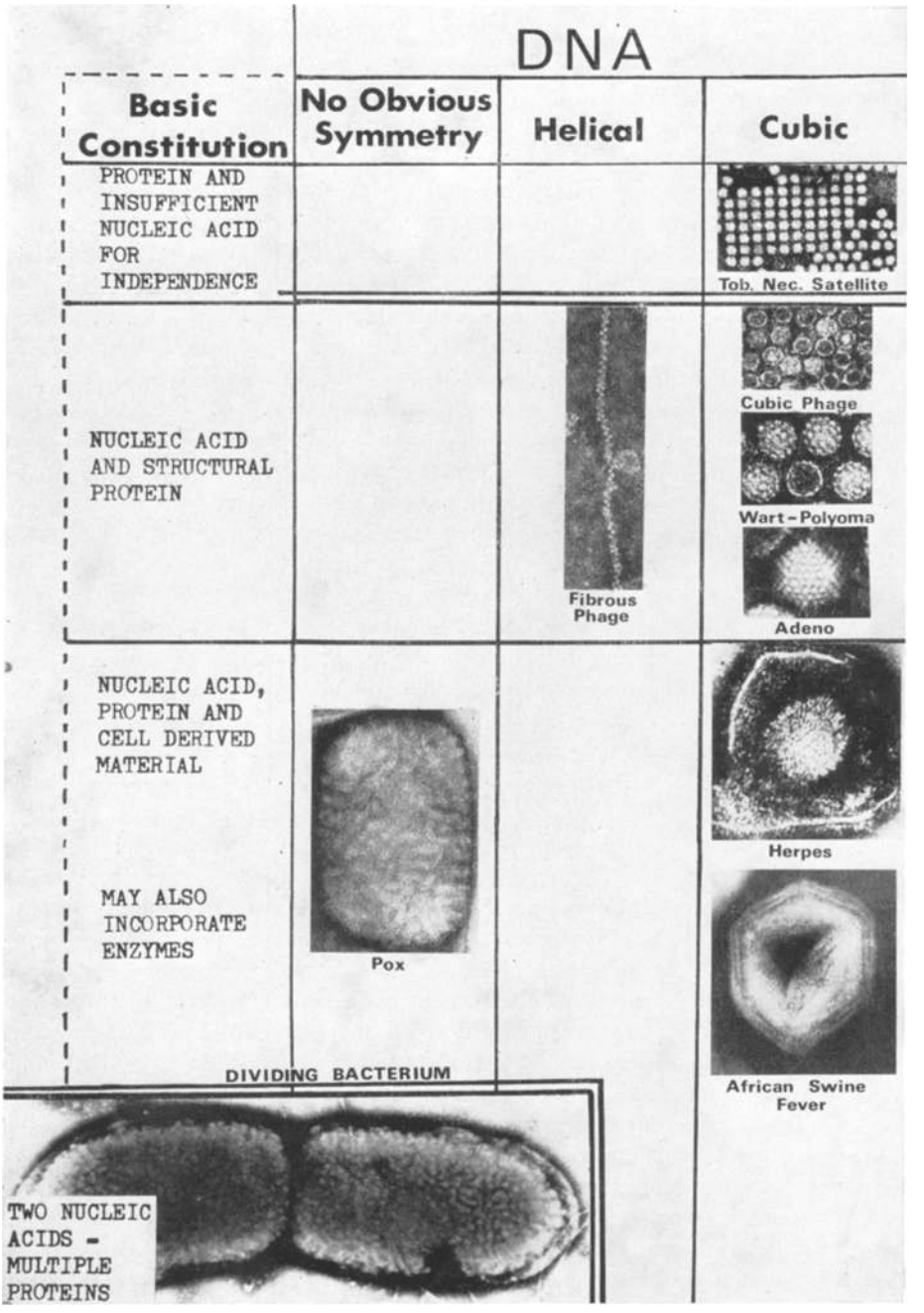
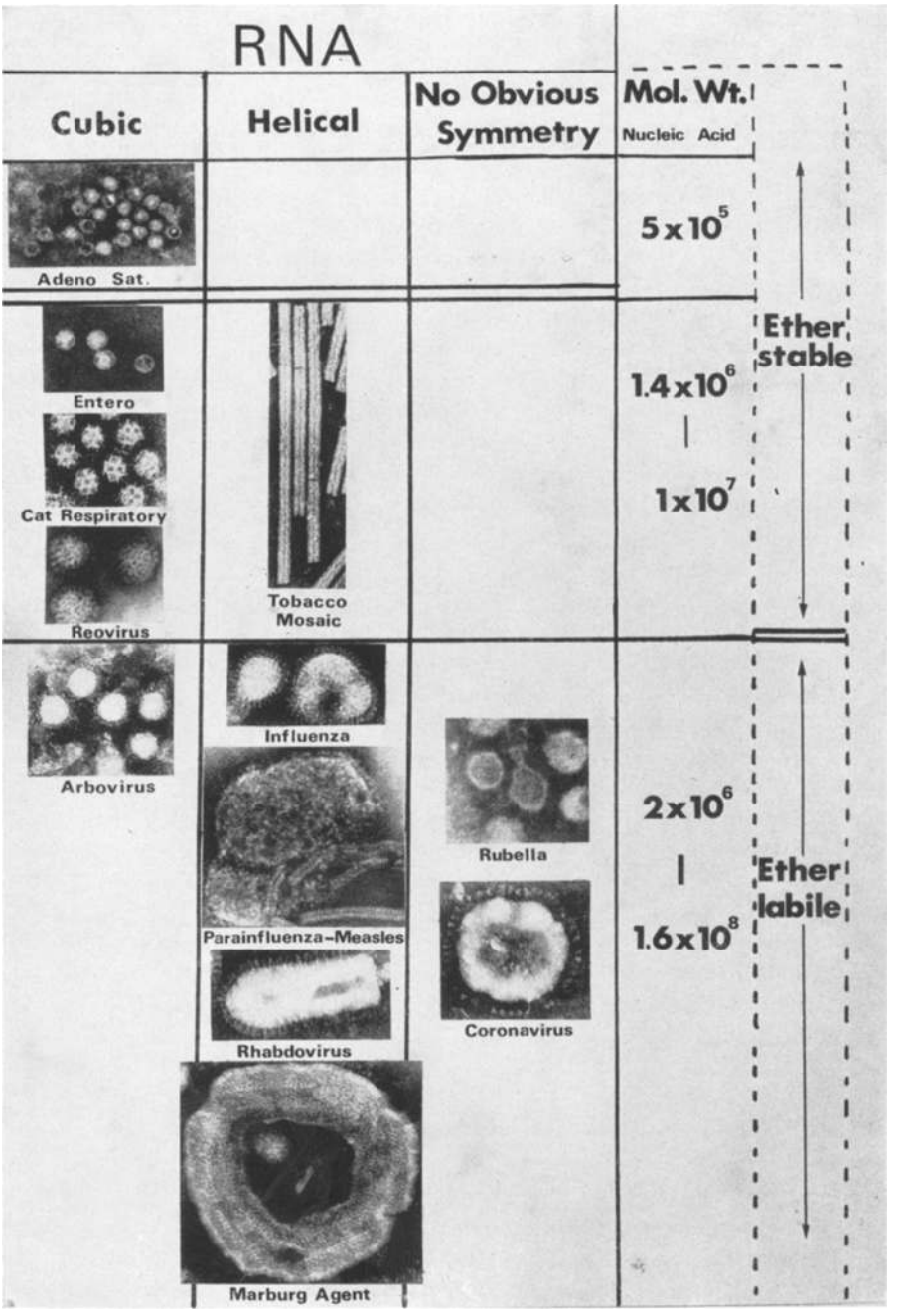


Fig. 1. A composite of micrographs of negatively stained virus particles showing the proposed arrangement. All of the virus particles are at the same magnification, 120,000 times. The bacterium is 2/3 this magnification, being 80,000 times


RNA
Cubic
Helical
No Obvious Symmetry
Mol. Wt.
Nucleic Acid
Adeno Sat.
5x10^5
Entero
Cat Respiratory
Reovirus
Tobacco Mosaic
1.4x10^6
1x10^7
Ether stable
Arbovirus
Influenza
Parainfluenza-Measles
Rhabdovirus
Marburg Agent
Rubella
Coronavirus
2x10^6
1.6x10^8
Ether labile

viruses, which are unable to replicate without a helper virus even in susceptible cells (KASSANIS and NIXON, 1961), to the poxviruses at the other, which because of their size and complexity are sometimes suspected of not being true viruses at all. Data on viral nucleic acid contents are admittedly incomplete and often only approximate (BURKE, 1967). In some viruses only the type is known. Nevertheless there is sufficient data of adequate reliability for a general trend to be discerned when viruses are arranged in order of ascending nucleic acid content. Generally speaking, this coincides remarkably with the vertical arrangement into satellite viruses, simple viruses and compound viruses. Those above the ether line have lower nucleic acid contents than those below, although there is, not surprisingly, a certain area of overlap. The interesting conclusion emerges that at lower nucleic acid values symmetry appears to be obligatory, and above the ether line is no example of a virus not displaying obvious symmetry. However, as the amount of nucleic acid increases, the symmetrical components of the virus become concealed in cell-derived membranes, and beyond that, at the highest levels (poxviruses) obvious symmetry is no longer a part of the viral morphology. In other words the degree of frank symmetry varies inversely with the absolute nucleic acid content.

Almost inevitably in a discussion of basic virus properties one is led back to a consideration of what attributes make up a virus. For technical reasons the first serious work on virus structure was carried out by X-ray diffraction (HODGKIN, 1949), and the limitations of this technique confined it to selected viruses that could be obtained in sufficient quantity and purity. In practice this limited the study to the simple viruses above the ether line, and it is not surprising that in each case it was possible to demonstrate that the virus particles displayed either cubic or helical symmetry. Concurrently with this the nucleic acid content of viruses such as tobacco mosaic and turnip yellow mosaic became available. These figures, together with the X-ray diffraction results, led CRICK and WATSON (1957) to speculate on theoretical grounds that viruses contain so little nucleic acid that their protective protein covering would have to be made of repeating identical subunits and that these subunits must be arranged in a simple manner as regards each other, *i.e.* that they would display either cubic or helical symmetry. This then led to the postulate that a virus, in its physical make-up, would have a moiety of one type of nucleic acid and a symmetrically arranged layer of protein subunits to protect this essential genetic element. Obviously, this description is an apt one for all those viruses above the ether line, but is less fitting for those below it.

It seems then that ether-stability (or lability) produces an essential distinction between those viruses that might well be described as classic and the others that at least in terms of human disease make up the larger part of the virus kingdom. These viruses were originally termed compound, and this name still serves for purposes of discussion. A new consideration of the origin and biological status of this outer, cell-derived, membrane presents these compound viruses in a novel light. It seems that viruses below a certain level of nucleic acid content have insufficient genetic information to enable them to manipulate and incorporate the cell membrane while those better endowed can adapt and exploit this already existing structure for their own protection. Of course, organic solvents

are unlikely to be encountered in nature by viruses and the so-called ether-labile viruses in fact probably have as yet ill-understood biological advantages over their simple ether-stable counterparts. The poxviruses, with their still larger nucleic acid content, are able to synthesise their own lipid-containing coat rather than making use of the existing cell membrane. In other words, the higher its nucleic acid content, the more a virus departs from the classical concept of a virus. A dividing bacterium has been included at the bottom left hand corner of Fig. 1, and it will be readily apparent that the poxvirus illustrated has a certain, even though superficial, resemblance to it, and it should be pointed out that such a bacterium, in common with all organisms on this side of the ether line, is sensitive to organic solvents.

To sum up, if viruses are arranged primarily on the basis of morphology and composition, including a consideration of the amount of nucleic acid present, several interesting features appear. First, it is possible to draw an ether-stability/lability line and so produce a distinction between what might be termed the classical viruses and the rest. Second, it appears that the more nucleic acid a virus has the more it is freed from the strict dictates of symmetry. Also, at the upper margin of nucleic acid content, viruses have other features, such as sensitivity even to some conventional antibiotics (SUBAK-SHARPE *et al.*, 1969), and the possession of internal enzymes, features not encountered among the smaller, classical viruses. This means that the spectrum of viruses runs from the very small, strictly symmetrical, satellite viruses, which are incapable of replication without a helper virus, and which, on theoretical grounds, must represent the simplest possible form of life, to the large poxviruses, which are beginning to show some of the properties associated with the next higher groups of organisms, *i.e.* the Rickettsiae and the bacteria. Such a range of properties is to be expected within any group of organisms and, in this respect viruses seem to follow the usual pattern of having at one end a primitive and at the other end a sophisticated extremity. Also, as with other biological groupings, those members that would be described as displaying the classical features of the group belong in the middle of it, *i.e.* between the small defective satellite viruses and the large compound viruses. Finally, in the morphological arrangement put forward, it can be seen that only the upper part contains viruses adhering strictly to the rules of symmetry and property suggested for them. Clearly care must be taken that one part of the virus world is not taken for the whole.

Acknowledgments

We are indebted to Dr. B. Kassanis for the illustration of satellite tobacco necrosis virus. J. D. A. is supported by a grant from the Medical Research Council. A. P. W. acknowledges the support of Action for the Crippled Child (Polio Research Fund).

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