From the present X-ray evidence we are unable to distinguish the respective contributions of the protein and the ribonucleic acid, so we cannot be sure whether the cubic symmetry is perfect and applies strictly to both of them. We cannot tell whether the protein sub-units contain identical sequences of amino-acids, or whether the ribonucleic acid sub-units (if they exist) have identical sequences of nucleotides. It should not be very difficult, by end-group analysis, to decide whether the protein components are all approximately equal. By analogy with tobacco mosaic virus we would guess that this will be found to be the case. With the ribonucleic acid component, however, the problem is more difficult than it was in the case of tobacco mosaic virus, as the number of nucleotides per sub-unit is certainly much larger. (This follows from the higher percentage of ribonucleic acid[7] and the much smaller number of protein sub-units.) Only with a more detailed understanding of the ribonucleic acid core is the problem likely to be settled.

Animal and Other Viruses

For animal viruses we are handicapped because there is no X-ray evidence available so far. However, it is now becoming clear[2] that many of the smaller animal viruses, such as poliomyelitis and the various encephalitic viruses, are morphologically very similar to the spherical plant viruses. Not only are they of similar size (approximately 300 A. diameter); but it has recently been shown[18] that poliomyelitis virus also contains ribonucleic acid and can form crystals which appear as regular as those produced by the plant viruses. We thus think it very probable that cubic symmetry also extends to these animal viruses, and that the soluble antigens[19] (of about 120 A. diameter) frequently observed in infected cells are related to the sub-units normally used in the assembly of the final infective virus.

We also see no reason why our hypothesis should not be valid for viruses containing deoxyribonucleic acid rather than ribonucleic acid. Although the structure of bacteriophages is usually more complex than the smaller viruses discussed here, the fact that their heads appear polyhedral suggests that ideas of this general type may apply to them, too. On the other hand, it is less likely that they will be relevant to the structure of the larger viruses like vaccinia.

Conclusion

We can now describe our hypothesis in a more general manner. We assume that the basic structural requirement for a small virus is the provision of a shell of protein to protect its highly specific packet of ribonucleic acid. This shell is necessarily rather large, and the virus, when in the cell, finds it easier to control the production of a large number of identical small protein molecules rather than that of one or two very large molecules to act as its shell. These small protein molecules then aggregate around the ribonucleic acid in a regular manner, which they can only do in a limited number of ways if they are to use the same packing arrangement repeatedly. Hence small viruses are either rods or spheres. The number of sub-units in a rod-shaped virus is probably unrestricted, but for a spherical virus the number is likely to be a multiple of 12. Every small virus will contain symmetry elements and in favourable cases these can be discovered experimentally

We believe that this hypothesis is likely to apply (in this form or a simple variant of it) to all small viruses which have a fixed size and shape.

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Structure of Bushy Stunt Virus

Crystals of bushy stunt virus yield detailed X-ray diffraction patterns indicating a high degree of regularity in the virus structure. Bernal, Fankuchen and Riley[1] found that the unit cell is body-centred cubic with one virus particle per primitive lattice point. Previous X-ray photographs[1,2] did not show whether the symmetry as well as the shape of the lattice is cubic. This is of considerable interest, since cubic symmetry for the lattice would imply that the individual virus particles possess cubic symmetry[3]. This is discussed in the preceding article (by Crick, F. H. C., and Watson, J. D.).

Precession photographs have been obtained from single crystals of bushy stunt virus which were grown by Messrs. F. C. Bawden and N. W. Pirie and kindly supplied by Dr. C. H. Carlisle. Two of these photographs, showing the basal reciprocal lattice planes normal to the edge and body diagonal of the cubic unit cell, are illustrated in Figs. 1 and 2. These show two- and three-fold symmetry respectively about the cube edge and cube diagonal, and establish the space group as $I\ 23$ ($a = 386$ A.).

Early in this study, photographs were obtained from a disordered crystal which did not give lattice

reflexions but in which the relative orientation of the virus particles appears to have been maintained. In one particular orientation this crystal gave a pattern of diffuse reflexions lying in several rings, each of which contained ten symmetrically arranged spots. This suggests that the virus particle has five-fold as well as two- and three-fold symmetry.

If the virus has the point-group 532, rather than merely 23, it will have additional symmetry axes. There will be one set each of two-fold, three-fold and five-fold axes lying in non-crystallographic directions, as well as the set of two-fold and the set of three-fold axes belonging to the space group. The directions of members of each of these five sets are shown in Figs. 1 and 2, except for the three-fold axes of the space group which can be seen in another photograph not reproduced here.

The intensity distribution which such symmetry is likely to produce on an X-ray photograph is best considered by reference to the Fourier transform of an individual virus particle. It can be shown that this transform is likely to exhibit spikes of high intensity extending from the origin along the direction of the symmetry axes. The X-ray pictures actually confirm the existence of such spikes along the postulated axes. The only difficulty in the identification arises from the fact that the symmetry of the crystal lattice (23) is lower than that of the individual virus particles (532). This means that directions in the transform which are related by the symmetry of the virus particle have to be sampled on reciprocal lattice points which have no symmetry relation, and the arrangement of which tends to obscure or confuse the underlying symmetry of the transform. However, allowing for this sampling difficulty, it is found that the radial variation of intensity near one type of diad of the point groups 532 is very similar to that near the other type; this is exemplified especially by regions of zero intensity occurring at the same distance from the origin along both types of dyads. A similar correspondence is also found for the two types of triads. Moreover, the intensity variations along the dyads and triads are different.

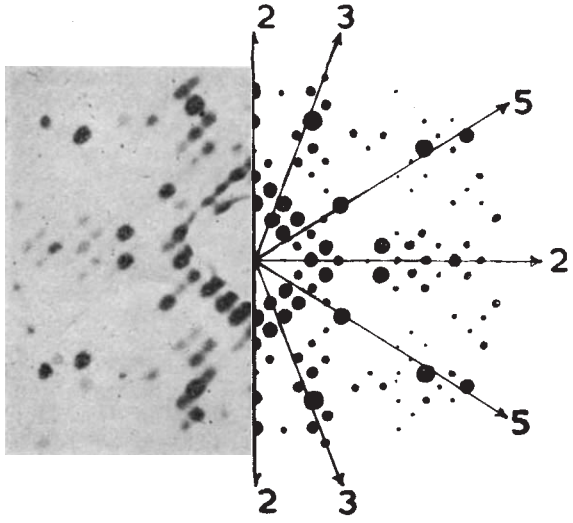

Fig. 1. Precession photograph of basal reciprocal lattice plane normal to the cube edge of bushy stunt virus crystal (left). Weighted reciprocal lattice net showing the orientation of the two- three- and five-fold axes of the point group 532 which lie in this plane (right). The two-fold axes in this plane lie along the [100] directions of the lattice

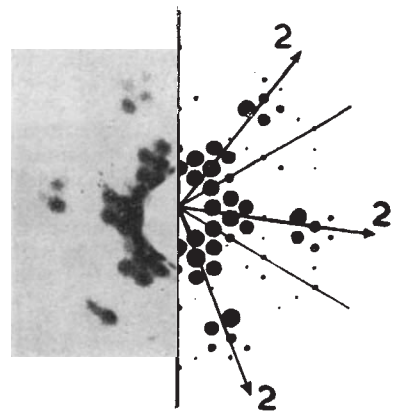

Fig. 2. Basal reciprocal lattice plane normal to cube diagonal showing the orientation of the two-fold axes of the point group 532 which lie in this plane. The unlabelled lines lie along the [110] directions of the lattice

All the evidence indicates that the virus has the point-group symmetry 532, which implies that it is built up of sixty structurally identical asymmetric units. The molecular weight of bushy stunt virus is about 9 million[4], of which about 17 per cent is ribonucleic acid[5]; therefore, the molecular weight of the protein part of the asymmetric unit is about 125,000. The point group symmetry does not preclude the possibility of each crystallographic unit being subdivided further into a number of chemically identical protein molecules. Thus 125,000 is a maximum value for the size of the protein sub-unit.

Chemical evidence, in fact, does suggest that the protein sub-unit may perhaps be smaller than this. There have been no end-group analyses reported for bushy stunt virus, but its amino-acid analysis obtained by de Fremery and Knight[5] can be used to calculate the minimum size of the sub-unit. Tryptophan, cysteine, methionine and histidine are present in very small amounts and approximately in the molar ratio 1 : 2 : 2 : 3. Assuming that all the protein molecules in the virus are identical (which may not be true) these analyses give a minimum molecular weight of 25,000-28,000, suggesting perhaps three hundred protein sub-units.

Thus the X-ray evidence shows that bushy stunt virus possesses sub-units. The number of sub-units is certainly a multiple of twelve and very probably a multiple of sixty. The chemical data suggest that the actual number may be as high as three hundred.

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