THE STRUCTURE OF TURNIP YELLOW MOSAIC VIRUS: X-RAY DIFFRACTION STUDIES

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INTRODUCTION

The small viruses are the only nucleoproteins which have been obtained in crystalline form, and therefore provide the best available system for studying, by means of X-ray diffraction, the *in vivo* structure of protein and nucleic acid and the structural relationship of the one to the other. There is now a good deal of evidence (summarised in reference[1]) that, in the small viruses which consist only of protein and nucleic acid, the protein is in the form of identical or near-identical sub-units of rather low molecular weight. This was first established for the rod-shaped tobacco mosaic virus, where it has been shown[2] that the protein sub-units are arranged helically; that is, in crystallographic terms, the sub-units are related to one another by a screw axis, non-integer in this case. Generalising from this example, CRICK AND WATSON[1] put forward the hypothesis that the small "spherical" viruses, too, have protein sub-units related by symmetry elements, so that the same packing contacts between sub-units are used over and over again in building up a shell of protein. They pointed out that the most likely way of doing this is to use cubic symmetry, and this was confirmed by CASPAR[3] for the case of tomato bushy stunt virus.

We report here the early stages of an X-ray diffraction study of crystals of turnip yellow mosaic virus (TYMV). This has shown that the virus particle has cubic symmetry, with a strong pseudo-symmetry higher than that of the cubic unit cell of the crystal, and has given an indication of what the actual arrangement of the protein sub-units might be. A brief summary of this work has already been published[4].

Turnip yellow mosaic virus was first isolated and crystallised by MARKHAM AND SMITH[5]. It has a molecular weight of about 5 million, contains about 40% of ribonucleic acid, and is approximately spherical in shape, with an external radius of 140 A[6,13]. In infected plants there occurs, together with the virus, a closely related non-infectious protein, the so-called "top component". This contains no nucleic acid and has a molecular weight of about 3 million. This protein particle is also nearly spherical, having about the same external diameter as the complete virus, and MARKHAM suggested[6] that it had the approximate form of a hollow spherical shell. This was confirmed by SCHMIDT *et al.*[7] by means of low-angle X-ray scattering studies on solutions. (See also ref.[13]).

Both the virus and the nucleic acid-free protein can be crystallised from ammonium sulphate solution[5]. X-ray powder photographs of the crystals were first taken by BERNAL AND CARLISLE[8], and later, when larger crystals became available, "still

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photographs[9]" of single crystals were obtained[10]. It was shown that the unit cells of both the virus and the protein were cubic, and of sides 703 A and 715 A. BERNAL AND CARLISLE suggested, on account of the absence of the 222 reflection, that there were 8 particles in the unit cell, and that these were arranged like the carbon atoms in diamond. This appeared to agree with the electron micrographs obtained by COSSLETT AND MARKHAM[11] of dried micro-crystals, which showed an open structure characteristic of a diamond-type lattice. The results presented here are, however, incompatible with this type of structure and have led us to propose a new crystal structure for the virus. It will be seen that this new structure can be considered as made up of two diamond-type lattices which interpenetrate in such a way that the centres of the virus particles lie on a single body-centred cubic lattice.

THE CRYSTAL STRUCTURE

Some fairly well-formed crystals of the virus, about 0.3 to 1.0 mm in diameter, prepared by Dr. MARKHAM in 1948, were kindly given to us by Dr. CARLISLE. MARKHAM also supplied us with a preparation of small crystals of size 10–20 μ of the "top component", which was suitable for powder work.

Using a Hilger semi-microfocus X-ray unit and a Buerger precession camera, we have obtained precession photographs of single crystals of the virus, showing reflections at spacings down to about 20 A. The intensities on the photographs fall off rapidly with angle, but we have been able to observe some reflections at smaller spacings, and even a few at 4 A. Three of these photographs, taken with the X-ray beam approximately parallel to the cube edge and the face- and body-diagonals respectively, are reproduced in Figs. 1a, b, and c.

The symmetry of the photographs shows that the lattice symmetry is cubic, and the systematic absences establish the space group as $F4_13$. If virus particles are located at lattice points it follows that the particles themselves must have cubic symmetry; that is, they must have at least the point-group symmetry 23. If, on the other hand, the particles lie in general positions in the unit cell, then their symmetry cannot be established from a knowledge of the space group alone. This latter possibility can, however, be eliminated by a consideration of the number of virus particles in the unit cell.

It is not always possible to determine this number from X-ray diffraction data alone. However, when the particles (or atoms or molecules) lie at special points in the unit cell the arrangement is usually revealed by absences in the X-ray diagrams not required by the space group. In the case of TYMV two systematic sets of such absences are observed, and from these it is possible to deduce both the number and arrangement of the particles in the unit cell. Firstly, it is observed that, at low angles, reflections having h, k, l, all odd are absent or very weak (see Fig. 1b). Secondly, for spacings out to 20 A, reflections having $h + k + l = 4n + 2$ are not observed; at spacings between 20 A and 10 A they are either absent or too weak to observe (this is shown on "still" photographs taken in the [111] direction).

These absences, taken in conjunction with the space-group absences, mean that, at very small angles where the virus particles may be expected to scatter approximately as spheres, the only reflections observed are those with $h + k + l = 4n$. These are characteristic of a unit-cell which has a side of length half that of the true unit

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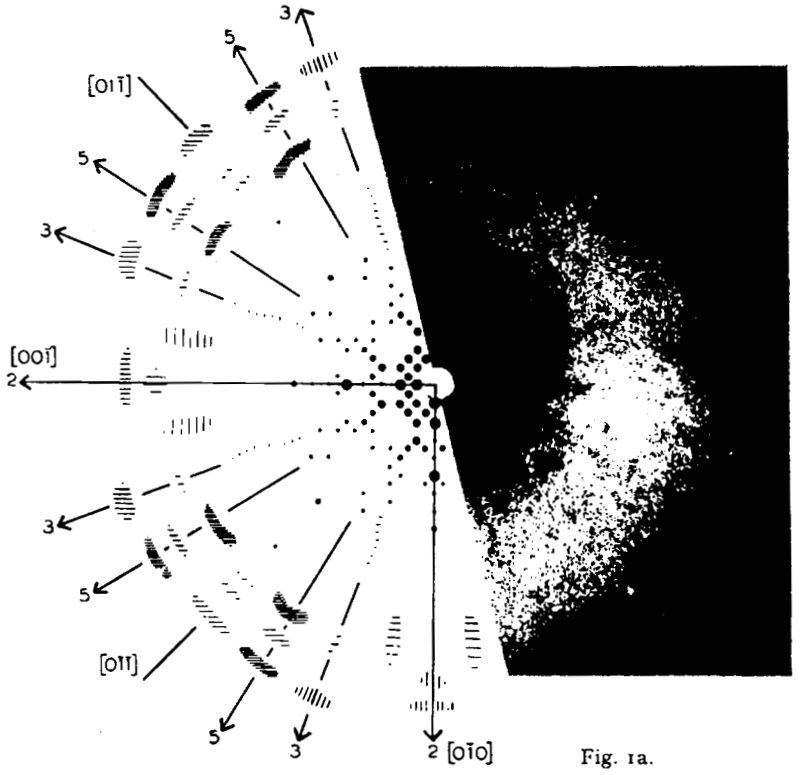
3
5
[01$\bar{1}$]
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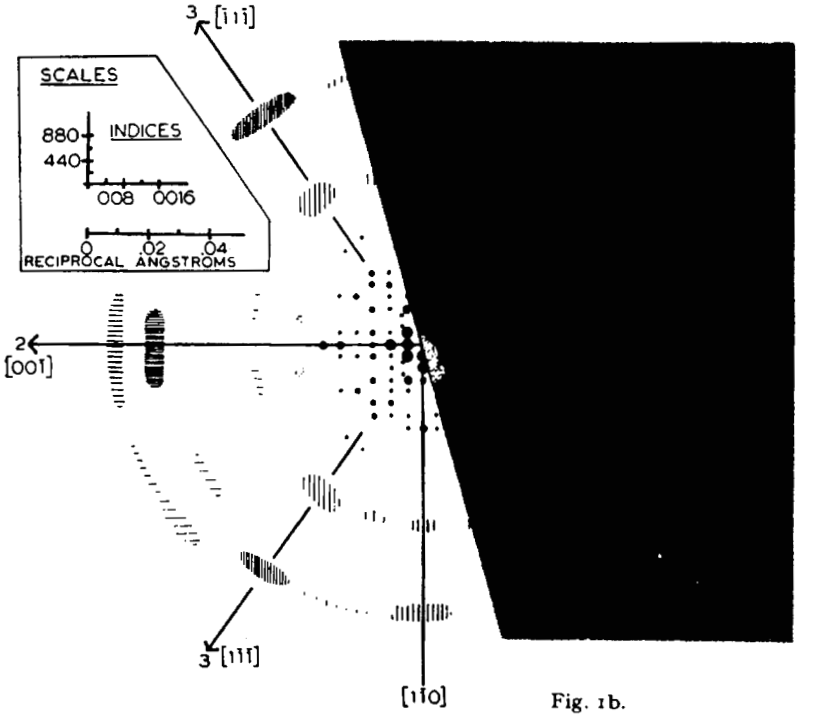
Fig. 1a.


3 [$\bar{1}\bar{1}\bar{1}$]
SCALES
880
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008 0016
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Fig. 1b.

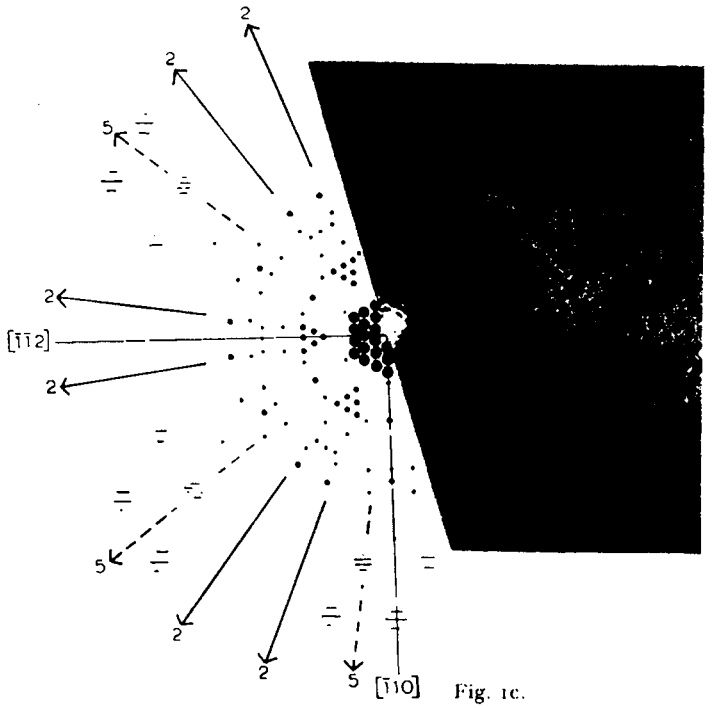

Fig. 1c.

Fig. 1. Precession photographs of a crystal of turnip yellow mosaic virus taken without a layer-line screen with the X-ray beam parallel to: (a) a cube edge [100], (b) a face diagonal [110], (c) a body diagonal [111]. The photographs are all reproduced on the scale indicated in (b). In each case the beam has been set slightly off the axis to show more of reciprocal space. The precession angles are: (a) 1°20′; (b) 1°20′; (c) 2°; parts of several reciprocal lattice layers are visible in each photograph. Schematic representations of the photographs are shown in the adjoining diagrams, the intensity of a reflexion being indicated roughly by the size of the dot. Diffuse regions are indicated by hatching. Owing to geometrical factors, a reflexion does not always occur with the same intensity on different photographs or different parts of the same photograph. The positions of the 2-, 3-, and 5-fold rotation axes lying in each of the basal reciprocal lattice planes are indicated by full-line arrows; the number of positions occurring is doubled with respect to the point-group symmetry because of the 4-fold symmetry of the crystal. In 1(c), 5-fold axes lying a little out of the basal (111) plane, to one side of it, are indicated by their projections (dotted lines) on to this plane. Strong intensities occur near the intersection of these axes and non-zero layers of the reciprocal lattice.

cell, and which is body-centred. Thus, viewed at low resolution, the crystal appears to have a simple body-centred cubic unit cell of side about 350 A, and with one particle per lattice point. Since such a cell contains 4 particles, the true unit cell of side 700 A must contain 16. The crystal structure of TYMV thus resembles that of tomato bushy stunt virus[3] in that the *centres* of the particles are located at the lattice points of a simple body-centred cubic lattice. The larger size of the true unit cell in turnip yellow mosaic virus must be associated with the occurrence of particles *in more than one orientation* (see below).

Confirmation that crystals of TYMV contain 16 particles per unit cell of side 700 A has been obtained by means of ultra-violet absorption measurements carried out with the assistance of Dr. P. M. B. WALKER[12] of King's College, London. Absolute measurements of the ultraviolet absorption per unit length of crystal were compared

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with the known absorption of the virus in solution. In this way it was shown that the unit cell contains 18 ± 3 virus particles. Because the space group $F4_13$ requires a multiple of 8 particles per unit cell, the correct value must be 16.

We have thus shown that, as in bushy stunt virus, the particles of turnip yellow mosaic virus lie at the lattice-points of a cubic unit cell; it follows that the particles themselves must possess cubic symmetry. CRICK AND WATSON[1] have shown that the only possible cubic point-group symmetries which can occur in virus particles are 23, 432 and 532. It is impossible to arrange 16 virus particles having 432 symmetry in a large unit cell with space-group symmetry $F4_13$, for then the distinction between the two orientations would disappear, and so the cell side would be halved. Hence the virus particle must have 23 symmetry, with the possibility that the additional, non-crystallographic symmetry of 532 is also present.

We have explored systematically all possible ways in which particles having 23 or 532 cubic symmetry might be set in different orientations at the lattice points of the cubic body-centred pseudo-unit cell of side 350 A, in order to produce a true unit cell of cubic symmetry and side 700 A. We find that there is only one such arrangement which preserves both the cubic symmetry and the size of the large unit cell. In this arrangement alternate particles lying along the direction of the cube edge occur in two orientations at 90° to each other in the manner shown schematically in Fig. 2. It will be seen that, not only does this arrangement give a cubic unit cell of the required size, but it also has the observed space group $F4_13$.

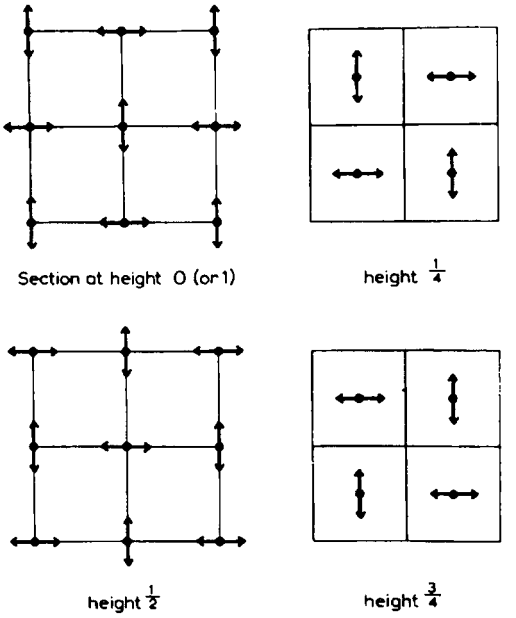

Fig. 2. The arrangement of 16 virus particles in the unit cell. Sections at cell heights 0 (or 1), ¼, ½ and ¾ are shown. The two different orientations of the virus particles are indicated, each particle being represented by a double-headed arrow.

We may now refer again to the two classes of systematic non-space group absences, the observation of which led us to propose a structure of this type. It may be shown that the first class, having $h + k + l = 4n + 2$, is *strictly* absent in the structure proposed. On the other hand reflections having h, k, l, all odd are absent only at spacings so large that the two different orientations of the particle cannot be distinguished from one another. This is consistent with our observations. Whereas no reflection having $h + k + l = 4n + 2$ has been recorded, reflections having h, k, l, all odd are observed at large angles, and also, very weakly, close to certain reflections of very large structure factor at lower angles.

The argument for the proposed crystal structure may be re-stated in very general terms as follows. The condition that reflections with $h + k + l = 4n + 2$ are absent means that every fourth plane in reciprocal space perpendicular to a [111] direction is absent. The most general arrangement in real (crystal) space that could account for this is that which has the equally spaced sequence ....*AABBAABB*.... along the body-diagonal of the unit cube, where *A* and *B* denote

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different scattering factors. Since all virus particles are the same* the only possible difference between *A* and *B* is one of orientation. Now if there were only 8 particles in the unit cell the sequence would have to be ...*AA*...*AA*...*AA*... (*i.e.* in the above notation $B = 0$), but this is not consistent with the space group. We conclude that the sequence along the cube diagonal is indeed ...*AABB*..., and, as can be seen from Fig. 2, this is just the structure we have proposed.

INTER-PARTICLE CONTACTS IN THE CRYSTAL

In a body-centred lattice, nearest-neighbour contacts are along the direction of the 3-fold axis. For the particular arrangement of particles, of symmetry 23 or 532, shown in Fig. 2, the nearest-neighbour contacts are of more than one kind. If the opposite ends of a 3-fold axis in any particle be labelled *l* and *r*, then inter-particle contacts may be classed as *ll*, *rr*, or *lr* (= *rl*). For the point-group 532, *ll* and *rr* are equivalent; for 23 they are different. In the structure which we have described each particle forms 4 contacts of type *lr* and 4 of type *ll* or *rr*. Another way of stating this is that the 8 nearest neighbours of any one particle all turn the same kind of face (either *l* or *r*) towards it.

It should be noted that other body-centred lattices can be constructed in which *only lr* or *only ll* and *rr* contacts are used, but in each case the unit cell contains only 4 particles (*i.e.* cube side half of that which we observe). A possible (but not the only) explanation of the more complicated arrangement which leads to the large unit cell in turnip yellow mosaic virus is that second-nearest-neighbour interactions are important.

THE SYMMETRY OF THE VIRUS PARTICLE

The crystal structure described above requires that the virus particle have 23 symmetry, and excludes the possibility of its having symmetry 432. We must now consider whether, in addition to the 23 symmetry, the non-crystallographic symmetry of the point group 532 is also present.

CASPAR has presented evidence of 532 symmetry in tomato bushy stunt virus[3]. Our photographs of turnip yellow mosaic also show strong indications of 532 symmetry. In Figs. 1a and 1c we have indicated the directions of those 2-, 3-, and 5-fold axes of the point-group 532 which are not used in constructing the crystal lattice. It will be seen that there is a concentration of high intensities around these directions, indicating at least a pseudo-symmetry of type 532. This effect is somewhat less obvious for turnip yellow mosaic virus than for tomato bushy stunt, owing to the extra symmetry of the crystal of the former, which causes a doubling of the number of spikes of high intensity (see Fig. 1). It seems probable, however, that 532 symmetry is present to about the same extent in the two cases. In turnip yellow mosaic virus there is the further complication that, although the ⟨110⟩ directions are not parallel to any rotation axes of the individual particles, they are, however, parallel to two-fold screw axes of the crystal cell as a whole, with the result that the intensity tends to be strong in these directions also.

That 532 symmetry is not *fully* present in the turnip yellow mosaic virus is shown

* We can probably safely exclude the possibility of both right-handed and left-handed forms of the virus particle existing simultaneously. In any case it is highly unlikely that these would be present in a crystal in equal numbers and regularly alternating in the manner shown in Fig. 2.

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by the presence of weak but distinctly visible 333 and 555 reflections, as well as by BERNAL AND CARLISLE'S observation of a 111 reflection. It can be shown that reflections of the type *hhh* with *h* odd would be strictly absent for the structure proposed if the *true* symmetry of the particles were 532, since this is a non-polar point-group in which positive and negative directions of the 3-fold axis are equivalent.

The existence of 23 symmetry in the virus particle implies that it is built up of 12, or a multiple of 12, asymmetric sub-units. If the particle had true 532 symmetry (of which 23 symmetry forms a sub-group) at least 60 asymmetric sub-units would be required.

In considering the arrangement of sub-units in turnip yellow mosaic virus it is important to remember that the ribonucleic acid content of the virus is high; it is responsible for about a half of the total X-ray scattering. True 532 symmetry would therefore require 60 sub-units in both the nucleic acid and the protein parts of the virus. The fact that the virus shows only a high degree of pseudo-symmetry of the 532 point-group may be accounted for in one of two ways. The first possibility is that, whereas both protein and ribonucleic acid have 23 symmetry, the higher 532 symmetry is fully developed only in one of the two components, *i.e.* it is only partially present in the virus particle as a whole. From a consideration of the genetic function of ribonucleic acid one might expect that it would contain a smaller number of sub-units than the protein, and hence that 532 symmetry might be present in the protein and absent or only partially present in the nucleic acid.

Alternatively, it is possible that neither the protein nor the nucleic acid has strict 532 symmetry, but that there is an arrangement of sub-units simulating this symmetry. This could come about if, say, the protein were in the form of 60 sub-units, not all identical, but with their centres placed according to 532 symmetry. Pseudo-532 symmetry of this kind could also arise if there were only 12 sub-units of protein or nucleic acid arranged at the vertices of an icosahedron, but here the effect on the X-ray diagram would be weaker, and so this possibility seems unlikely since the spikes of intensity extend clearly as far out as 10 A. Indeed we have been able to identify a small number of strong reflections in the 5 A region lying very close to the 5-fold symmetry axes. It is highly improbable that this could arise from an arrangement of sub-units which did not have true 532 symmetry in at least one of the two components.

THE NUCLEIC ACID-FREE PROTEIN PARTICLE

The question of whether or not the virus *protein* has full 532 symmetry, and therefore a multiple of 60 sub-units, could best be answered by studying diffraction by single crystals of the nucleic acid-free virus protein particles. Crystals of the protein large enough for the purpose have not yet been obtained; we have, however, been able to obtain a certain amount of relevant information from a study of X-ray *powder* photographs given by preparations of small crystals of the protein. These were taken using crystal-monochromatised CuKα radiation and a high-resolution focusing camera. The work represents, essentially, an extension to much larger angles of the measurements made by SCHMIDT *et al.*[7] on the virus protein in solution. In the angular region common to their measurements and ours the results are in good agreement when allowance is made, in our case, for the sampling effect due to the presence of a crystal lattice.

We thus confirm that, seen at low resolution, the protein particle approximates to

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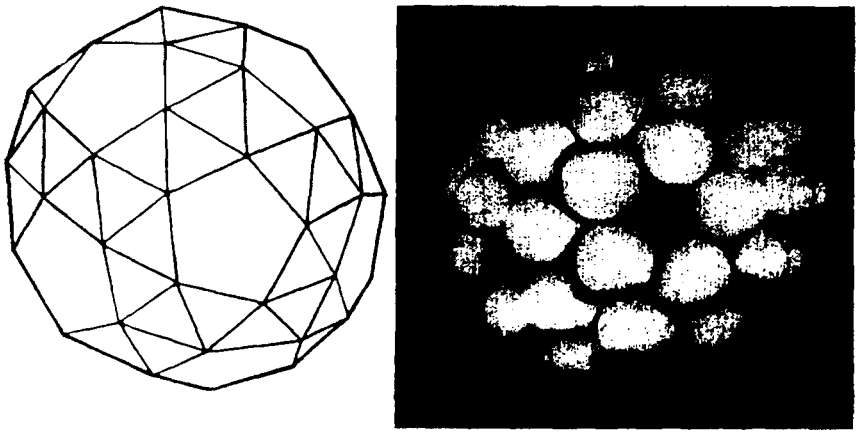
a hollow sphere, with internal and external radii about 105 A and 140 A. At somewhat higher angles, the powder diagram deviates from the theoretical intensity for a hollow sphere, and shows a distinct intensity modulation, of period about $1/60$ A^{-1}, indicating a predominant spacing of about 60 A in the protein particle. We would expect that this represents the distance between protein sub-units, and must therefore look for an arrangement of sub-units which would give such a spacing. We have done this, and find that, if each protein sub-unit is represented by a sphere of diameter 60 A, and if 60 such spheres are placed with their centres at the vertices of a snub dodecahedron (a semi-regular solid having the symmetry 532, see Fig. 3a) of such a size that each sphere is in contact with its five nearest neighbours, then the *equivalent spherical shell* of the snub dodecahedron has internal and external radii about 105 A and 140 A. These are the same as the values deduced above for the X-ray scattering at low angles. (Dr. F. H. C. CRICK had in fact earlier suggested to us that this was a likely arrangement, consistent with 532 symmetry, for 60 protein sub-units, since of all such possible arrangements it is the most densely packed.) A model of a "spherical" shell made up of 60 sub-units having this arrangement is shown in Fig. 3b.



(a) (b)

Fig. 3. (a) A snub dodecahedron. This is a semi-regular polyhedron possessing 532 symmetry. It has sixty vertices, six 5-fold axes, ten 3-fold axes and fifteen 2-fold axes. (b) Suggested model of a "spherical" shell of protein made up of 60 sub-units, lying at the vertices of a snub dodecahedron. For convenience in building, a sub-unit is represented by a ping-pong ball; the sub-units could, however, be perfectly general in shape, and all of them would still be in equivalent positions. The model is placed in approximately the same orientation as the diagram in (a).

A more formal way of looking at the same data is to calculate the radial Patterson function (Fourier transform of the observed intensity curve) and compare it with theoretical Patterson functions for various arrangements of spheres (representing protein sub-units). In this way we find that agreement with the snub dodecahedron described above is good for nearest neighbours but not beyond. Further work is required to see whether the discrepancies are due to the experimental error in measuring intensities at such high spacings, as is possible, or whether the protein shell is, in fact, made up of an arrangement of 60 sub-units, rather resembling the snub dodecahedron, but different in detail (the small rhombicosidodecahedron, for instance).

Thus our results suggest that there are 60 approximately spherical protein sub-

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units of diameter 60 A, each at the vertex of a snub dodecahedron and in contact with its nearest neighbours. We would emphasise, however, that this suggestion is based on indirect evidence from powder diagrams only, and measurements on single crystals must be made before any such model can be considered proved. Symmetrical arrangements of fewer than 60 spherical sub-units would all have inter-sub-unit distances significantly greater than 60 A. We have not, however, fully investigated possible structures built up from non-spherical sub-units.

COMPARISON WITH ELECTRON MICROSCOPE OBSERVATIONS

We must now discuss the relation of our conclusions from the X-ray work to the more direct picture of the external morphology of the virus particles obtained by means of the electron microscope. Electron micrographs of TYMV have been obtained by KAESBERG[14] and by STEERE[15]. It will be more convenient to discuss the latter work first.

STEERE's photographs show a rather regular arrangement of knobs on the surface of the virus particle, but these knobs are spaced about 90 A from their nearest neighbours, and there appear to be only about 12 of them per particle. Moreover, the most frequent arrangement of the 12 knobs is apparently that which has 432 symmetry (*i.e.* the vertices of a cube-octahedron) and this is incompatible with 532 symmetry. Only a relatively small number of particles can be seen on which 12 knobs appear to be arranged with 5-fold symmetry (*i.e.* at the vertices of a regular icosahedron).

There is thus an apparent discrepancy between our results and the structural features shown by these very high quality electron micrographs. We can only conclude that X-ray diffraction and electron microscopy are in some way showing different aspects of the turnip yellow mosaic virus structure. We would note, however, that although the X-ray diffraction method is the more indirect, it also carries less possibility of the introduction of artefacts. In particular, the virus crystals contain ammonium sulphate to the extent of about 50% by weight of the dry virus, and this may well be of primary importance in the electron micrographs. In our model for the protein part of the virus (Fig. 3b) there are 12 holes at the positions of the 5-fold axes and these are about 90 A apart. If the ammonium sulphate crystallised out in these positions, the number and spacing of the knobs in STEERE's electron micrographs would be explained. The discrepancy in the observed symmetry would, however, remain.

KAESBERG[14], on the other hand, has concluded, from a study of the shadows cast by TYMV particles, that the virus particle has the approximate shape of a regular icosahedron. Thus, in respect of symmetry, KAESBERG's observations agree with our X-ray results, but the actual shape would not at first sight appear to be consistent with our model. It should be remembered, however, that the X-ray results refer to the whole of the virus particle, while the electron micrographs may possibly be emphasizing some relatively minor surface feature. The sub-units might, for instance, be so shaped as to give rise to an external icosahedral appearance.

CONCLUSION

We have shown that turnip yellow mosaic virus crystallises in the cubic space group $F4_13$, with 16 particles in a unit cell of side about 700 A, the particles being orientated

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as shown in Fig. 2. The virus particles themselves must have at least the symmetry of the cubic point-group 23. The distribution of intensities in precession photographs of single crystals of the virus indicates that they have a strong pseudo-symmetry of the point-group 532. Powder diagrams of the nucleic acid-free virus protein particles provide indirect evidence that the protein part of the virus possesses 532 symmetry, the protein being in the form of 60 sub-units (or groups of sub-units) of about 60 A diameter, possibly lying at the vertices of a snub dodecahedron.

The powder diagrams also confirm the value of 140 A for the external radius of the virus particle, established by other workers. The work, however, shows that this value refers only to the *mean* (square) radius. On the model of a snub dodecahedron the maximum distance from the centre is about 150 A for spherical sub-units and could be more for differently shaped sub-units. It is then no longer necessary to postulate free water between the particles in the crystal (*cf.* refs.[7] and [8]). The (hydrated) virus particles are probably in contact along the 3-fold axes. The changes on drying would then result either from shrinkage of the virus particles themselves, as suggested by MARKHAM[6], or from some degree of interlocking (*cf.* the phenomena observed in the case of tobacco mosaic virus[16]).

The detection of the pseudo character of the 532 symmetry in the complete virus particle is possible because of the symmetry of the large crystallographic unit cell. It should be noted that in bushy stunt virus[3], which has a simple small unit cell, there is no immediate way of determining whether the 532 symmetry is fully or only partially present.

We hope to be able to obtain in the near future single crystal precession photographs of the ribonucleic acid-free virus protein. From these it should be possible to establish with more certainty the arrangement of protein sub-units in the virus, and also, from a detailed comparison of the nucleic-acid-free protein and the complete virus, to determine the location and sub-unit arrangement of the ribonucleic acid.

ACKNOWLEDGEMENTS

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SUMMARY

X-ray diffraction studies of crystals of turnip yellow mosaic virus and of the associated protein particles have led to new results concerning (a) the packing of the virus particles in the crystal cell, and (b) the arrangement of sub-units in a virus particle.

In the virus crystals the particles lie with their centres at the lattice points of a body-centred cubic unit cell of side 350 A, but are not all in the same orientation so that the true crystallographic cell has a side of 700 A and contains 16 particles.

The virus particle as a whole has point-group symmetry 23, and is therefore built up of 12, or a multiple of 12, sub-units. The additional symmetry of the point-group 532 is also partially present. It is concluded that there are probably 60 sub-units forming the protein "shell" of the virus.

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