An Electron Microscope Study of the Structure of
Sericesthis Iridescent Virus

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SUMMARY

Purified suspensions of Sericesthis iridescent virus were treated with a
nasal decongestant, negatively stained and examined in the electron micro-
scope. The outer icosahedral surface of the virus particles showed morph-
ologica subunits apparently in close-packed hexagonal array and 70 Å apart.
The orientation of untreated particles was determined from their outline.
This made it possible to measure precisely their icosahedral edge-length
(86 ± 27 Å).

Prolonged storage of purified virus in distilled water at 4 ° resulted in
disintegration of the particles into triangular, pentagonal and linear frag-
ments. The triangles of side 700 Å were clearly composed of 55 hexagonally
arrayed subunits 70 Å apart. Edges of the pentagons appeared to consist of
three subunits also about 70 Å apart, while the linear fragments had a broad
length distribution about a mean of 438 Å.

All these observations, interpreted by a new approach to the ‘Goldberg
diagram’, suggested that the virus surface is composed of 1562 morphological
subunits, though alternatives of 1292 and 1472 subunits cannot be excluded.

INTRODUCTION

Various large icosahedral viruses (around 1500 Å diameter) form iridescent pellets
when centrifugally sedimented. The first was isolated from Tipula paludosa (Xeros,
1954) and others have since been reported in Sericesthis pruinosa (Steinhaus & Leu-
tenegger, 1963), in Aedes taeniorhynchus (Clark, Kellen & Lum, 1965) and in Chilo
suppressalis (Fukaya & Nasu, 1966). Smith & Hills (1960) have claimed that the surface
of Tipula iridescent virus particles (TIV) is composed of 812 morphological subunits.
Mercer & Day (1965) reported that the particles of Sericesthis iridescent virus (SIV)
were ‘indistinguishable in size and shape’ from particles of TIV, though they found
no evidence for the presence of any subunits on the surface of SIV. However, all
agree that both viruses are icosahedral in shape (Williams & Smith, 1958; Steinhaus &
Leutenegger, 1963).

This paper describes electron microscopic studies of the outer icosahedral shell of
SIV, which was found to consist of many more than 812 morphological subunits.
There are many ways in which these subunits could be arranged on the icosahedral
surface, but I describe here why three possible arrangements seem more likely than
the others.
METHODS

Preparation of purified suspensions of SIV. The virus was extracted from infected larvae of the wax moth *Galleria mellonella* kindly supplied by Dr T. D. C. Grace, C.S.I.R.O., Canberra. The larvae had been infected with SIV 3 years previously by the method of Day & Mercer (1964) and stored deep-frozen. They were ground with a small quantity of 0.01 M-borate buffer, pH 7. The large impurities were sedimented by slow centrifugation and the supernatant fluid containing virus in suspension was centrifuged once more (27,000 g, 20 min.), giving the typical iridescent pellet. The clear supernatant fluid was discarded and a layer of impurities covering the pellet washed gently off with more borate buffer. The pellet was covered with buffer and resuspended by storing overnight at 4°. The virus was further purified by resedimenting (12,000 g, 30 min.), washing off a further layer of impurities and finally resuspending in distilled water. Examination of this suspension in the electron microscope showed very little material other than intact virus particles.

Electron microscopy. Virus preparations were mounted on carbon films supported on 400-mesh copper specimen grids. They were negatively stained by mixing with an equal volume of either 4 or 1 % aqueous solution of sodium silicotungstate at pH 7 and examined in a Philips EM 200 electron microscope. Electron images were recorded on Ilford N.50 plates.

Preparation of virus particle fragments. When a virus preparation is made alkaline (pH 11 or higher), the particles are disrupted but only small amorphous fragments are formed. However, when purified virus was stored in distilled water at 4° for long periods it apparently became labile, for when stained and dried on the electron microscope grid some triangular arrays of subunits (triangles) and other fragments were found. One preparation contained triangles after only a few weeks’ storage (Pl. 1 a), whereas another preparation contained fragments only after about 12 months’ storage (Pl. 1 b).

Treatment of intact virus particles to reveal surface structure. Numerous physical and chemical treatments failed to make the surface structure of SIV visible in various negative stains. However, Mercer & Day (1965) suggested that SIV particles may be coated with mucopolysaccharide, which would effectively prevent the penetration of negative stain between subunits. I therefore diluted a sample of virus suspension with an equal volume of a nasal decongestant (‘Afrin’, Schering Corp., New Jersey, U.S.A.). The ingredients of ‘Afrin’ per ml. distilled water are oxymetazoline hydrochloride 0.5 mg., glycine 3.8 mg., sorbitol 40.0 mg., phenylmercuric acetate 0.02 mg., benzalkonium chloride 0.2 mg., adjusted to pH 5.5 to 6.5 with sodium hydroxide. SIV was also treated with these ingredients separately, but with no perceptible effect. After 48 hr at room temperature about 10 % of the virus particles showed surface detail (Pl. 2 a). However, I do not understand the success of this treatment, for the principal action of ‘Afrin’ is said to be vaso-constrictor and not muco-solvent! (Schering Corporation, personal communication).

Calibration of electron micrograph magnifications. All specimens to be measured were mounted and photographed in the electron microscope with crystals of bovine liver catalase (Pl. 2 b) in a manner similar to that suggested by Luftig (1967). The 86 Å lattice spacing of these crystals provided each electron micrograph with its own internal standard of length accurate to within ± 2.5 % (Wrigley, 1968).
a Three fields showing 'triangular' fragments of SIV, with their 55 subunits clearly resolved.
b The three types of SIV breakdown fragment. The 'triangular' fragments are obvious, some 'pentagonal' fragments are ringed (see also Pl. 4b), and some 'linear' fragments are arrowed.

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(Facing p. 124)
a Eight selected examples of 'Afrin'-treated SIV particles showing hexagonally close-packed morphological subunits. On the basis of outline, the upper row are thought to be in threefold projection, the bottom row in twofold projection.

b A typical field of SIV particles with those labelled 2, 3 and 5 identified as being in two-, three- and fivefold projection respectively. Arrows show examples of a surface ‘fringe’ of fibres. Lower right: portion of a calibrating catalase crystal.

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Plate 3

a, b, c Sheet Perspex model of an icosahedron X-rayed along two-, three- and fivefold axes of symmetry respectively.
d, e, f Selected examples of three distinctive ‘membrane’ outlines of SIV particles.
g Particles of adenovirus showing random orientation on the electron microscope grid.

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a Collapsing particles of SIV. Left: early stages of collapse showing ‘triangles’ and ‘pentagons’ still in their original positions (cf. Fig. 4). Centre: the same picture with the interpretation of structure superimposed. Right: another particle at a later stage of collapse.

b Three selected fields each showing a ‘pentagonal’ SIV fragment with three subunits per edge of the pentagon (cf. Fig. 3c). Their size may be gauged from the ‘triangles’ in the central field.

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All measurements were made directly on the original photographic plates. Images of the crystal patterns (at least 100 lattice repeats on each crystal) and the triangular SIV fragments were measured with a Gaertner two-dimensional comparator. The intact virus particles and linear fragments were measured with a Bausch and Lomb spectrum magnifier graduated in 0.1 mm. intervals.

**RESULTS AND DISCUSSION**

**Intact particles of SIV**

**Rough estimate of structure**

The particles of SIV are clearly icosahedral in shape. Various physical and chemical treatments before electron-microscopic examination failed to reveal their surface structure though a fringe of irregular fibres was often seen projecting from the surface (some examples arrowed, Pl. 2b). However, after treatment with ‘Afrin’ morphological subunits could be seen on the surface of some particles (Pl. 2a). The centre-to-centre distance between these subunits ranged from 60 to 69 Å. The orientation of these particles could not be determined, so that the largest measurement (69 Å) is likely to be best, being least foreshortened.

Hosaka (1965) suggested an approximate method for evaluating $N$, the total number of morphological subunits per particle, from this distance between subunits and the maximum observed particle diameter $D$. For intact particles of SIV, $D$ was found to be about 1700 Å. Hence Hosaka’s method gives $N = 1672$, about double the figure of 812 proposed for TIV by Smith & Hills (1960). This approach does not satisfy the criteria of Caspar & Klug (1962), that ‘... an icosahedral arrangement [of subunits] cannot be regarded as established until (1) at least two neighbouring 5-coordinated morphological units can be unequivocally identified, and (2) the arrangement of 6-coordinated morphological units in their neighbourhood can be discerned’. It seems improbable that these criteria ever will be met for such a large virus particle with some 1700 subunits. Certainly, not even one 5-coordinated subunit, let alone two neighbouring ones, can be seen in images like those in Plate 2a. However, the occurrence of regular surface fragments of SIV suggested an alternative way of determining the structure of this virus. But before the fragments and whole particles could be compared their dimensions had to be accurately measured.

**Measurement of intact particle edge-length**

Estimates of the ‘diameters’ of icosahedral virus particles, such as those of SIV, usually vary greatly (e.g. Mercer & Day, 1965, obtained a variation of ±13%). Much of this variation arises because the orientation of particles on the electron-microscope grids is not known, so that estimates include the ‘face-to-face’ diameter and the ‘corner-to-corner’ diameter, in itself a ±11.5% difference. Therefore before particles can be measured accurately their orientation must be determined.

Some negative stain usually penetrated the outer shell of SIV particles, so that this shell appeared as a ‘membrane’ surrounding a central core (Pl. 2b). This membrane was indistinct and irregular in the majority of particles, but in a significant minority one or other of three distinctive morphological types was seen. Those particles labelled 2 (Pl. 2b) have a particularly prominent pair of opposite corners and their adjacent four sides, the remaining two sides being very faint. In those particles labelled 3 all
six sides are equally prominent and they form a regular equilateral hexagon, unlike the particles labelled 2. Those particles labelled 5 have an almost circular outline, in contrast to the other hexagonal outlines. As an analogue of the virus shell's electron image, a model icosahedron made of Perspex sheet was X-rayed in a manner suggested by the work of Almeida, Waterson & Fletcher (1965) and Caspar (1966). Plate 3a, b and c shows images of this model X-rayed along axes of two-, three- and fivefold symmetry respectively; Plate 3d, e and f shows the three distinctive appearances of the SIV 'membrane' for comparison. There is an obvious similarity between the pairs of pictures a d, b e and c f so it seems that the three appearances of SIV correspond to particles viewed along their two-, three- and fivefold axes of symmetry.

Fig. 1. (a), (b) Outline of SIV particles in two- and threefold projection respectively, indicating the dimensions actually measured in each case.

This was confirmed in a different way. If we assume that SIV particles can adopt any orientation when drying down on the electron microscope grid, then the numbers of particles identifiably in two-, three- and fivefold orientation will be in the ratio 30:20:12. This assumption of random orientation is reasonable, and is borne out by particles of adenovirus whose subunits are easily resolved (Pl. 3g). When electron micrographs of several hundred SIV particle images were examined, the orientation of over half the particles (490) could not be determined. Of the remainder, 197 were in twofold, 136 in threefold and 61 in fivefold orientation, a ratio of 300:207:93. Thus the counts of two- and threefold projections were indeed in the expected proportions, though the count of fivefold projections was lower than expected. This confirmed the identification of at least the two- and threefold oriented particles (Fig. 1, a and b respectively), so these particles were measured in order to calculate the edge-length L of the icosahedral shell. For these purposes L is defined as the distance between centres of subunits situated on vertices of the icosahedron. It was found easier and more reproducible to make measurements to just outside the 'membrane'; lengths x₁ and x₂ (Fig. 1a) were measured and recorded when equal (i.e. when the particle was exactly in twofold projection) and lengths y₁, y₂ and y₃ (Fig. 1b) were likewise recorded when equal. From such measurements on 50 particles the edge-length was calculated (see Appendix A) to be

\[ L = 860 \pm 27 \AA, \]  

where the uncertainty of 3·1 % is the standard deviation. The smallness of this standard deviation further supports the identification of orientation, for it includes not only
the maximum uncertainty of 2.5% to be expected from the catalase calibration, but also an uncertainty of 1.4% due to a maximum possible departure of 10° from exact two- or threefold projection (see Appendix B).

Fragments of SIV particles

Triangles. After some weeks' storage in water at 4° a purified suspension of SIV which originally contained only intact particles was found to contain triangular arrays of subunits ('triangles'). Most of these had ten subunits per edge, i.e. 55 subunits per triangle (Pl. 1a). Some triangles were damaged in an irregular fashion but if the missing units were replaced the triangle would always have had ten units per edge. The distance between rows of subunits was measured using the comparator, and from this the centre-to-centre distance \( l \) between subunits was calculated. The mean value for 50 triangles was calculated to be

\[
l = 70.1 \pm 2.3 \text{ Å},
\]

where the 3.3% uncertainty is the standard deviation. This result agrees well with the maximum reading of 69 Å measured on intact particles treated with 'Afrin'.

Other fragments. After storage in water at 4° for about 12 months another purified suspension of SIV showed even larger numbers of triangles (Pl. 1b). In this preparation two additional kinds of fragment were found. Many of the first kind (some are ringed in Plate 1b) appeared pentagonal in outline, but their shape was often too vague to make size measurements worthwhile. However, some of them showed three subunits per edge of the pentagon (Pl. 4b) and their over-all size may be gauged from triangles present in the same field. The other kind of fragments (some are arrowed in Plate 1b) appeared to be fibres roughly equal in length to the triangle edge. Five hundred and fifty such fibres were measured in terms of the edge-length (10 \( l \) over-all) of triangles also present in the field. The result was a very 'flat' normal distribution of fibre lengths about a mean of 6.25 \( l \) (438 Å). The standard deviation of \( \pm 2.0 \) \( l \) (140 Å) shows that the maximum fibre length is of the same order as the triangle edge-length.

The occurrence of these three kinds of fragment indicates the presence of at least three different proteins in the virus coat, each having a different quaternary structure (triangle, pentagon or fibre in the case of SIV). In fact A. J. Gibbs, W. G. Laver and I (unpublished results) have found at least ten different proteins in disrupted SIV preparations by electrophoresis in polyacrylamide gels. We do not know which of these are coat proteins and which internal proteins.

Deduction of the SIV particle structure

The triangles are presumably derived from the shell of the SIV particle; the subunits in the triangles look like those in the 'Afrin'-treated particles and the distance between them is almost the same. However, whereas the edge-length of the triangles is 700 Å, that of the intact particles (vertex to vertex) is 860 Å. This suggests that each particle does not degrade into 20 triangles alone, but that other fragments are produced. Before discussing the fragments, however, I will show that the probable number of subunits in the whole particle can be more accurately estimated from the measurement of \( L \) and \( l \) above.

The Goldberg diagram. The problem of finding all possible ways of arranging units in hexagonal close packing on an icosahedral surface (pentagonal packing at vertices) was solved by Goldberg (1937). His ingenious graphical method has been used by
Horne & Wildy (1963), Caspar (1964) and others to describe the structure of some simple icosahedral virus particles. I will discuss this method in detail, as the subunits of SIV seem to show this kind of hexagonal close-packing. Fig. 2 is an amplified version of Goldberg's diagram; the circles can be regarded as representing the positions of morphological subunits and are plotted on axes of \( a \) and \( b \) inclined at 60°. If the circle at the origin represents a subunit at one vertex of the icosahedron, then any other circle may be taken to represent a second vertex. Together they define an edge of some particular icosahedron, the circles in the region between them showing the arrangement of other subunits on the icosahedron. The number in the circle at the second vertex is the total number \( N \) of subunits on that particular icosahedral surface. Values of \( N \) are generated by the expression

\[
N = 10 \left( a^2 + ab + b^2 \right) + 2,
\]

where \( a \) and \( b \) may take any positive integral values. \( N \) is therefore quantized; its values shown in the diagram are the 'quantum numbers' of this type of virus architecture with hexagonal close-packing of morphological subunits. (Note that the value \( N = 1672 \) derived for SIV by Hosaka's method is not among them). The values of \( N \) shown are repeated by reflexion of the diagram in the median line \( a = b \); circles on the lines \( a = 0, a = b \) and \( b = 0 \) define the non-skew arrangements while the remaining circles define the skew arrangements with enantiomorphs on opposite sides of the line \( a = b \).

Some established examples of virus structures are: (a) adenovirus represented by the circle at \((5, 0)\) having 252 subunits with four subunits between vertices, (b) herpes virus represented by the circle at \((4, 0)\) having 162 subunits with three subunits between vertices, (c) papilloma virus represented by the circle at \((2, 1)\) and (d) turnip yellow mosaic virus represented by the circle at \((1, 1)\).

In the Goldberg diagram the distance from the origin to the centre of some particular circle, say at \((a, b)\), is \( \sqrt{a^2 + ab + b^2} \), and represents the edge-length \( L \) of a virus with a vertex at \((a, b)\) in terms of the distance \( l \) between subunits. That is:

\[
L = l \sqrt{a^2 + ab + b^2}.
\]

Thus a virus with a vertex at \((5, 0)\) (adenovirus) has an edge-length \( L = 5l \); a virus with a vertex at \((7, 4)\) has an edge-length \( L = l \sqrt{93} = 9.65l \), etc., the multiplier of \( l \) in each case being read off on the \( a \)-axis.

**SIV and the Goldberg diagram.** The converse of the above argument is that a virus will have a vertex somewhere on the circular arc of radius \( L/l \), centre \((0, 0)\). \( L \) and \( l \) have been determined experimentally for SIV (results 1 and 2), and their ratio is

\[
L/l = 12.25 \pm 0.8.
\]

An arc of radius 12.25 units is shown in Fig. 2 with further arcs one standard deviation (0.8) on either side. These define a region on the diagram within which a circle representing a vertex of SIV should lie. This is seen to narrow the field of alternatives for SIV to a choice of about a dozen different arrangements. However, the choice may be reduced still further by considering the fragments produced by the virus particle. An icosahedron has 30 axes of twofold symmetry, 20 of threefold symmetry and 12 of fivefold symmetry. Therefore the subunits on the surface of an icosahedral virus may be thought of as divided into 30 identical groups, each having twofold symmetry, 20 groups with threefold and 12 groups with fivefold symmetry. For these groups
Fig. 2. The Goldberg diagram showing the possible arrangements of hexagonally close-packed units on the surface of an icosahedron. The origin of axes represents a unit at one vertex, and any other circle a second vertex. The number in the upper half of each circle is the value of *N* for that particular arrangement. The value of *d* is shown in the lower half of each circle. Circles in any particular vertical column have the same value of *t* as plotted at the top of the diagram. Horizontal rows of circles have the same value of *p* shown at the left, and the type of unit arrangement corresponding to each row is sketched at the right. In these sketches • represents a dissymmetron component, ◇ a trisymmetron component and ◆ a pentasymmetron component with × representing an icosahedral vertex at the centre of each pentasymmetron.
I propose the names 'disymmetron', 'trisymmetron' and 'pentasymmetron' respectively, and one of various ways of constructing them is shown in Fig. 3 (c), (b) and (a) respectively. If \( d \), \( t \) and \( p \) are respectively the number of subunits in each kind of group, then their values are quantized as indicated, so that \( 30 \cdot d + 20 \cdot t + 12 \cdot p = N \). For example, adenovirus in this scheme would consist of thirty \( d = 4 \) disymmetrons, twenty \( t = 6 \) trisymmetrons and twelve \( p = 1 \) pentasymmetrons. In fact Laver et al. (1968) have reported non-triangular \( t = 9 \) trisymmetrons for adenovirus, and Valentine & Pereira (1965) have found \( p = 6 \) pentasymmetrons. Nevertheless the scheme proposed here, in which disymmetrons are linear, trisymmetrons triangular and pentasymmetrons pentagonal, seemed most relevant to the problem of SIV since fragments with these shapes have been found. In any case, this scheme adds to the use of the Goldberg diagram as a 'ready reckoner' of virus shell structures with hexagonal close packing of subunits, since values of \( d \), \( t \) and \( p \) may be plotted on it as in Fig. 2, giving an immediate indication of the type of structure for a given \( N \). Note that the cases where \( N \) is duplicated (e.g. there are two circles with \( N = 1472 \)) correspond to two different arrangements of subunits.

To return again to the structure of SIV, choosing one solution from the alternatives in the 'SIV experimental region' of Fig. 2 depends on unequivocal identification of all three types of symmetron. The triangles certainly can be identified as \( t = 55 \) trisymmetrons, and there are nine circles in Fig. 2 with this value of \( t \). The pentagons clearly seem to be pentasymmetrons, but their value of \( p \) is less certain. If they have three subunits per edge like those in Pl. 4b then \( p = 16 \), though very few such clear pentagons were seen. The majority showed no substructure but in many there were

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Fig. 3. Diagrammatic illustration of one of the ways in which an icosahedral array of close-packed subunits may be thought of as divided into pentagonal, triangular and linear groups ('symmetrons'). (a) Pentasymmetrons, having \( p \) subunits per group; (b) Trisymmetrons, having \( t \) subunits per group; (c) Disymmetrons, having \( d \) subunits per group. The quantized values of \( p \), \( t \) and \( d \) are shown on the right, where \( n = 1, 2, 3, 4, ... \)
three subunits on at least one edge. Their over-all size is too great for them to have only two subunits per edge, but four subunits per edge \((p = 31)\) is possible. Whether or not the linear fragments arrowed in Pl. 1\(b\) should be identified as disymmetrons is uncertain. None of them showed subunits, and their length distribution suggests that \(d\) may have any value up to about 10, including zero. Thus of the nine alternative structures mentioned earlier with \(t = 55\), there are three most likely solutions of the SIV shell:

Fig. 4. Diagram of the proposed structure of the outer SIV shell composed of 1562 morphological subunits (handedness arbitrary). Trisymmetrons \((t = 55)\) are shown in white subunits, disymmetrons \((d = 9)\) in black and pentasymmetrons \((p = 16)\) in grey. The geometrical edges of the icosahedral are picked out in heavy broken lines.

(1) R. W. Compans (personal communication) pointed out to me that in Pl. 1\(a\) and in similar micrographs not published here some of the triangles lie with their edges touching each other. Such edges do not lie corner to corner but most are ‘out of register’ with one another by three subunits. This observation suggested firstly that these
triangles remained joined in this way as the virus particle disintegrated, and secondly
that \( d = 0 \). Thirdly, \( p = 31 \) pentasymmetrons would be required to complete the
icosahedral shell. Thus \( t = 55, p = 31 \) and \( d = 0 \) give \( N = 1472 \) corresponding to
the circle at (7, 7) in Fig. 2. However some contiguous triangles were found to be out
of register by other than three subunits; moreover \( p = 31 \) seems less likely than
\( p = 16 \) (see Pl. 4b). Therefore the \( N = 1472 \) solution for the SIV shell structure seems
less likely than solutions (2) and (3) below.

(2) If it is accepted that \( p = 16 \), then there are two solutions with \( t = 55 \). One of
them again has \( d = 0 \), giving \( N = 1292 \). This structure is not very likely for two
reasons; it is more than one standard deviation from the mean experimental arc for
SIV in Fig. 2, and the triangles would have to be ‘bent’ over the icosahedral edges,
yet they always appeared flat in the micrographs.

(3) The other solution with \( p = 16 \) and \( t = 55 \) has \( d = 9 \), giving \( N = 1562 \). This is
about three times closer to the mean experimental arc in Fig. 2 than the \( N = 1292 \)
solution. Also it does not require the triangles to be ‘bent’. The fact that \( d = 9 \),
requiring fibres of length 9 \( l \), is consistent with the observed fibre lengths to the extent
that their maximum length is of the right order.

Thus on balance the evidence seems to point to SIV having an icosahedral shell
structure with 1562 morphological subunits altogether* (triangulation number \( T =
156 \)), though the \( N = 1472 \) and \( N = 1292 \) solutions cannot be excluded. From the
Goldberg diagram the arrangement of these 1562 subunits must be as shown in Fig. 4,
though the handedness of this skew structure is unknown. Occasionally particles were
found which appear to be in the early stages of breakdown. One such particle (Pl. 4a)
closely resembles Fig. 4 in parts, giving some direct confirmation of the suggested
structure.

Similar structural studies are now in progress with *Tipula* and *Chilo* iridescent
viruses. So far the former has reacted to the ‘Afrin’ treatment, showing surface arrays
of subunits very similar to those of SIV (Pl. 2a).

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Classics in this University for their advice in naming the symmetrons.

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* This conclusion differs from a preliminary report of this work (Bellett, 1968) in which the
\( N = 1112 \) solution was preferred. That conclusion was based entirely on the observation of the
triangles and considerations of how to pack them most simply into an icosahedron.
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APPENDIX A

Calculation of the SIV edge-length

In the figure ABCDEF represents a section through a pair of opposite edges (AB and DE) of an icosahedron. These edges are defined as 2,000 units long, so that the other four sides of the figure will each be \( \sqrt{3} \) units long (medians of triangular faces). Then using the notation shown
from triangle AFK \((x/2)^2 + w^2 = 3\),
from line FKLC \(x - 2w = 2\).

Thus \(x = 1 \pm \sqrt{5} = 3.236\) units. Since AED and AMP are similar triangles, \(AM = (2.000/3.236)MP\). Also \(PQ = AB - 2AM\) so that

\[
PQ = 0.618(x - 2MP). \tag{1}
\]

The figure actually represents a twofold projection of an SIV particle and may be identified with Fig. 1a in the text, so that \(x = x_1 = x_2 (= y_2 = y_3 = y_3, \text{Fig. 1b})\).

Measurements on 50 SIV particles gave

\(x = 1461 \pm 42\ \AA\).

MP approximates well to half the centre-to-centre distance \(l/2\) between subunits, where \(l = 70.1 \pm 2.3\ \AA\) (result 2 in text). PQ is the edge-length \(L\) of the virus. Therefore substituting for PQ, MP and \(x\) in equation \(1\) above gives:

\[
L = 0.618 [(1461 \pm 42) - (70.1 \pm 2.3)] \AA
\]

\[
= 860 \pm 27 \AA.
\]

### APPENDIX B

The accuracy of estimating particle orientation

The text describes how the orientation of SIV particles in electron micrographs was determined. One hundred and ninety-seven particles were found in twofold, 136 in threefold and 61 in fivefold projection. The orientation of a further 490 particles could not be determined.

An icosahedron has 30 axes of twofold, 20 axes of threefold and 12 axes of fivefold symmetry. These are 62 ‘particular’ axes out of an infinitely large number of possible axes. Thus the chance of finding a particle in *exact* two-, three- or fivefold projection is infinitely small. Therefore the 394 particles so identified experimentally (out of the 884 particles counted altogether) must really have been within some small angular range \(\theta\) of the true projections.

Let the icosahedron be circumscribed by an imaginary concentric sphere of radius \(R\). If the icosahedron is tilted in all directions through angles up to \(\theta\) about its centre, then each of the 62 ‘particular’ axes sweeps out on the sphere a spherical cap of area \(2\pi R^2 (1 - \cos \theta)\). The area of the whole sphere is \(4\pi R^2\). Thus, provided the spherical caps do not overlap,

\[
\frac{\text{Area of 62 spherical caps}}{\text{Area of whole sphere}} = \frac{\text{No. of particles in ‘particular’ orientation}}{\text{Total no. of particles counted}}.
\]

That is

\[
\frac{62 \times 2\pi R^2 (1 - \cos \theta)}{4\pi R^2} = \frac{394}{884}.
\]

This expression gives: \(\theta = 9^\circ 45'\).

The 62 spherical caps subtending this angle at the centre do not overlap, and the maximum foreshortening due to this departure from exact ‘particular’ orientation is about 1.4%.

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