on 3- or 2-fold axes and 15 subunits on a 5-fold axis.

In view of the extraordinary uniformity of the center-to-center spacing of the majority of particles presented by Howatson (*4*) and Wildy *et al.* (*2*) and the evidence presented by Williams *et al.* (*5*), it seems probable that most of these uniformly spaced particles are in contact with one another. The center-to-center spacing of many of the uniformly spaced virus particles was measured as was the center-to-center spacing of those subunits which appeared near the center of individual viruses. The ratio, intervirus distance to intersubunit distance, was found to be 5.4 for the human wart virus and 5.9 for the polyoma virus. The same calculation was made for the two models assuming edge-to-edge contact of models viewed parallel to a 3-fold axis. These calculated values were 4.5 for the 42 subunit model and 5.7 for the 92 subunit model. These calculations imply that the 92 subunit model fits the observed structure better than the 42 unit model.

One possible explanation for the appearance of these viruses, assuming they have 42 morphological subunits, might lie in a severe distortion of the virus particle, in particular, flattening. However, this would render any attempt to interpret their substructure questionable.

This communication is not intended to prove that these four tumor viruses have the morphology of a 92-unit icosahedron, but rather to show that the 92 unit model is at least as compatible with the published micrographs as is the generally accepted 42-unit model. In fact, since 5 : 3 : 2 rotational symmetry is exhibited by a number of polyhedra, there is little evidence that these viruses have any sort of icosahedral morphology as implied by the subunit equation, $10(n-1)^2+2$ (*1*).

CARL F. T. MATTERN
Laboratory of Biology of Viruses, National Institutes of Health Bethesda 14, Maryland

References

1. J. L. Melnick, *Science* **135**, 1128 (1962).
2. P. Wildy, M. G. P. Stoker, I. A. Macpherson, R. W. Horne, *Virology* **11**, 444 (1960).
3. A. F. Howatson, *Science* **135**, front cover (23 Feb. 1962).
4. ———, M. G. Williams, J. D. Almeida, *Abstr. 5th Ann. Meeting Biophys. Soc.* (1961).
5. R. C. Williams, S. Kass, C. A. Knight, *Virology* **12**, 48 (1960).
6. C. F. T. Mattern, *ibid.* **17**, 76 (1962).

26 April 1962

Icosahedral Models and Viruses: A Critical Evaluation

Abstract. The evidence for a 42 capsomere structure for the capsid of the papova viruses and 92 for reovirus and wound-tumor viruses is corroborated. Some of the difficulties inherent in comparing large-scale icosahedral models with high-resolution electron micrographs of virus particles displaying cubic symmetry are discussed.

The symmetry of the virus capsid has been proposed as a fundamental property upon which an efficient system of virus classification might be based (*1*). To date all viruses exhibiting cubic symmetry have been found to possess the 5 : 3 : 2 (icosahedral) pattern. The number of morphological subunits (capsomeres) making up the capsids of these viruses has been found as a solution of the general equation

$$N = 10x(n-1)^2 + 2,$$

where N is the total number of capsomeres and x and n are integers (*1*). When $x = 3$ the solutions describe a number of triacontahedra. Only turnip yellows mosaic virus ($N = 32$, $n = 2$) has so far been found to belong to this series (*2*), but it is expected that a number of small plant viruses will follow suit. When $x = 1$ the general equation reduces to the form

$$N = 10(n-1)^2 + 2,$$

and the solutions pertain to a series of icosahedra where n is equal to the number of capsomeres on any one of the 30 edges. Virus capsids have been found where $n = 2$, 3, 4, 5, 6, and 10.

Recently, Melnick has chosen the existence of 42 capsomeres ($N = 42$, $n = 3$) as one of the criteria for grouping together a number of small tumorigenic animal viruses as the papova viruses (*3*). In the face of conflicting interpretations of morphological data obtained in a number of laboratories for certain members of this group (*4–6*) it would seem important at this time to examine some of our concepts of cubic symmetry as applied to these small viruses in an attempt to clarify the general situation and perhaps arrive at a more logical interpretation of the existing morphological data.

In our opinion the evidence for 42 morphological subunits on the capsids of polyoma, vacuolating SV-40, and the papilloma viruses is completely satisfactory (*7–9*), particularly when it is reviewed in conjunction with the recent findings for wound-tumor virus (*10*) and reovirus (*11*) (see Fig. 1, left), two viruses whose capsids have been found to consist of 92 capsomeres ($N = 92$, $n = 4$).

The central problem in identifying and classifying viruses by their basic symmetry and capsomere number revolves around our ability to determine confidently the number and arrangement of the morphological subunits which we see in high-magnification electron micrographs. It is important to work under conditions of maximum contrast and resolution. Huxley and Zubay (*2*) were aware of this problem and found that by printing negatively stained virus preparations in reverse contrast so that the capsomeres appeared as black instead of white spots, considerable gains in clarity could be obtained. Figure 2 illustrates this principle with SV-40, the simian papova virus. The micrograph in the upper right has been printed in reverse contrast, and it is a simple matter to identify a number of 5-fold axes of symmetry and to count most of the subunits. Application of a similar technique applied to the published micrographs of the Shope papilloma virus (*5*) and to micrographs prepared in our laboratory by K. O. Smith has located very clear axes of 5-fold symmetry and has established 42 capsomeres for the capsid of the rabbit papova virus.

Another problem is inherent in transposing to the submicroscopic level any observations carried out on large-scale models. The construction of symmetrical models from a number of identical rigid spheres can be very instructive (see Fig. 2) as long as one keeps in mind that such an approach is an oversimplification. Spherical building blocks may represent a close approximation to the actual subunits for viruses with small spherical subunits (for example, turnip yellows mosaic virus and enteroviruses), but they are inadequate substitutes for the hexagonally and pentagonally faced hollow columns of considerable depth, which are a closer approximation to the truth for the papova viruses (*7*). Suitably oriented models constructed from 30 hexagonal and 12 pentagonal columnar subunits reveal approximately 16 subunits around the periphery (*1*) (see Fig. 2, bottom right) while similar models composed of 92 such building blocks (80 hexagonal, 12 pentagonal units) reveal approximately 24 peripheral subunits (*1*) (Fig.

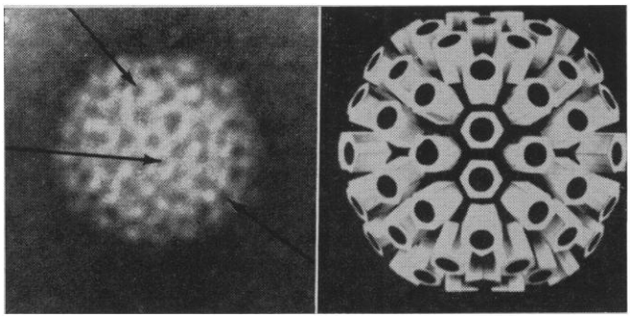

Fig. 1 (2 parts). (Left) High-resolution micrograph of reovirus from Jordan and Mayor (*11*). Arrows mark axes of 5-fold symmetry. $N = 92$, $n = 4$ (about × 340,000). (Right) Model from Horne and Wildy (*1*) built from 12 pentagonally faced and 80 hexagonally faced hollow columns.

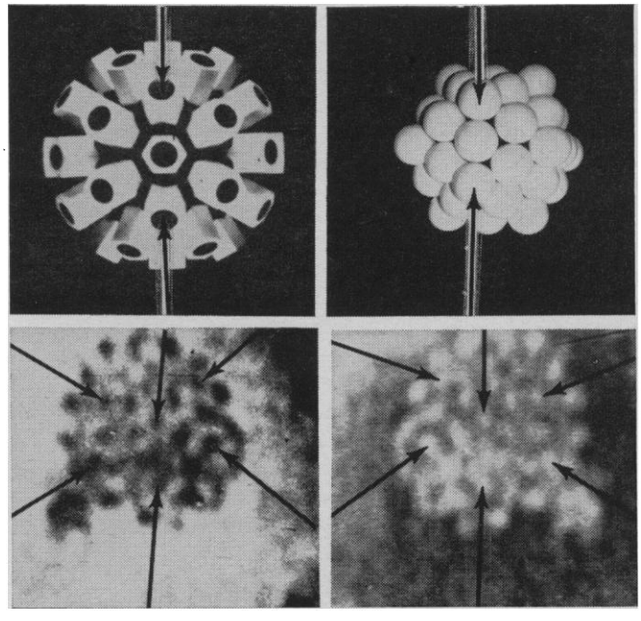

Fig. 2 (4 parts). (Top left) High resolution electron micrograph of simian papova virus SV-40 printed in normal contrast. (Top right) The same virus particle printed in reverse contrast (from Mayor, Jamison and Jordan, *9*). Arrows mark axes of 5-fold rotational symmetry. $N = 42$, $n = 3$ (about × 450,000). (Bottom) Forty-two subunit models built in accordance with the requirements of icosahedral symmetry. Model on left comprised of 42 rigid spheres viewed approximately along an axis of 3-fold symmetry. Model on right from Horne and Wildy (*1*) built from 12 pentagonally faced and 30 hexagonally faced hollow columns viewed along an axis of 2-fold symmetry.

1, right). Approximately 15 subunits can be located around the periphery of the negatively stained SV-40 virus particle (Fig. 2, top right), and 24 at the periphery of the reovirus particle (Fig. 1, left). These findings are in excellent agreement with the existence of 42 and 92 capsomere structures, respectively. Mattern's conclusion (*6*) that at most 12 subunits should be visible at the periphery of a 42-subunit model and 18 for a 92-subunit structure is based on models constructed from rigid spheres. His deduction that the capsids of the papova viruses could possibly be built from 92 subunits would appear to be incorrect. In addition, counts of subunits at the periphery of flattened virus particles are not satisfactory for arriving at the number of capsomeres making up the complete virus capsid. Once the basic cubic symmetry of a virus particle has been established as belonging to the icosahedral pattern by the detection of hexagonal profiles in electron micrographs, the regular occurrence of 5-fold axes of symmetry, or the typical appearance of double shadow-cast preparations (*12*), then it is only necessary in any suitable negatively stained preparation to locate two contiguous axes of 5-fold symmetry on favorably oriented particles and to count the number of subunits between them.

Figure 2 illustrates this principle and a value of $n = 3$, and hence $N = 42$ can be derived immediately for the simian papova virus. In this regard the objection that the edges of the virus particles may be submerged in a cloud of phosphotungstic acid and therefore that not all the capsomeres can be counted (*4*, *6*) is invalid, as a correct value can be arrived at as long as two favorable 5-fold axes can be discerned.

Confidence in identifying axes of 5-fold symmetry becomes of prime importance, and again prints made in reverse contrast facilitate this task. Distortions of specimen preparation may introduce considerable disorder in the finished electron micrographs. For example, in the papova virus model (Fig. 2, bottom left) two capsomeres marking axes of 5-fold symmetry are clearly shown. Very little deformation would be needed to confuse an observer so that he might consider each of these capsomeres to be surrounded by six rather than five similar subunits.

Visualization of approximately 30 subunits on the "top" surface of rabbit papilloma virus particles (*4*) is completely compatible with a value of $N = 42$ for the whole virus particle. Stability requirements demand that most virus particles with cubic symmetry align on the electron microscope grids so that we are looking along an axis of 3-fold symmetry (Fig. 2, bottom left and right, and Fig. 3). Under these conditions even a model constructed from 42 rigid spheres will reveal approximately 27 subunits (Fig. 2, bottom left), and with flattening and deformation an actual papova virus particle reveals at least 30 (Fig. 2, top right). The studies of Huxley and Zubay with turnip yellows mosaic virus (*2*) indicate that their technique usually reveals subunit structure on one surface of the virus particles only. In our experience with the papova viruses and reoviruses the finding of two superimposed sets of subunits was a common occurrence, particularly with material which had been banded in a cesium chloride isopycnic gradient. Al-

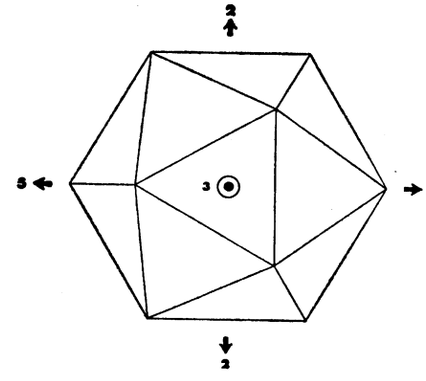

Fig. 3. Icosahedral plan of the rigid spherical model above showing axes of symmetry, as seen along an axis of 3-fold symmetry.

though the subunits on the "bottom" surface are usually less distinct, they may add to any estimation based solely on total counts of *visible* capsomeres in the negative.

Measurements on close-packed arrays of intervirus/intersubunit ratios (*6*), and their comparison with values obtained from models, have little meaning in the light of the parallax involved in measuring the spacings between long hollow capsomeres, and the assumption that all morphological subunits are identical in size and shape needs further consideration. While this may be nearly so for the papova viruses, there is strong evidence that the subunits of turnip yellows mosaic virus and the entero-viruses are much smaller than those of the papova group and the larger animal viruses (*13*).

It is common knowledge that in any biological species not all the members are perfect specimens and it can be expected that virus particles are no exception. Observation of negatively stained virus preparations in the electron microscope reveals that many virus particles are assembled more "symmetrically" than others. We must bear in mind that in any system of classification based on fine structure, the basic symmetry and capsomere number for a given virus species relates to the most morphologically favorable members in the preparation. It is not unlikely that during the course of construction of a virus capsid from an intracellular source of capsomeres, errors may occur and particles with variable numbers of subunits could be formed. Although we have seen many disordered particles in our papova virus preparations, none suggest a possible count of $N = 92$.

The evidence continues to mount that the papova viruses ($N = 42$, $n = 3$) are approximately 45 to 50 mμ in diameter and that their capsids consist of 30 hexagonally faced and 12 pentagonally faced hollow capsomeres. Next in the icosahedral series ($N = 92$, $n = 4$) come the reoviruses and wound-tumor viruses, approximately 60 to 70 mμ in diameter and possessing a capsid again with 12 pentagonally faced, but with 80 hexagonally faced columnar capsomeres. Proper interpretations of patterns of cubic symmetry are proving to be powerful tools in building new systems of virus classification (*14*).

HEATHER DONALD MAYOR
JOSEPH L. MELNICK
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

References and Notes

1. R. W. Horne and P. Wildy, *Virology* **15**, 348 (1961).
2. H. E. Huxley and G. Zubay, *J. Mol. Biol.* **2**, 189 (1960).
3. J. L. Melnick, *Science* **135**, 1128 (1960).
4. R. C. Williams, S. J. Kass, C. A. Knight, *Virology* **12**, 48 (1960).
5. C. Breedis, L. Berwick, T. F. Anderson, *ibid.* **17**, 84 (1962).
6. C. F. T. Mattern, *Science*, this issue, p. 612.
7. P. Wildy, M. G. P. Stoker, I. A. Macpherson, R. W. Horne, *Virology* **11**, 444 (1960).
8. A. F. Howatson, M. G. Williams, J. D. Almeida, *Abstr. 5th Ann. Meeting Biophys. Soc.* (1961); A. F. Howatson, *Science* **135**, front cover (23 Feb. 1962).
9. H. D. Mayor, R. M. Jamison, L. E. Jordan, in preparation.
10. R. F. Bils and C. E. Hall, *Virology* **17**, 123 (1962).
11. L. E. Jordan and H. D. Mayor, *ibid.*, in press.
12. R. C. Williams and K. M. Smith, *Biochim. et Biophys. Acta* **28**, 464 (1958).
13. A. Klug and J. T. Finch, *J. Mol. Biol.* **2**, 201 (1960); H. D. Mayor, in preparation.
14. The studies from our laboratory were aided by a grant from the National Foundation and by research grant C-4600 from the National Cancer Institute, National Institutes of Health.

19 June 1962

Preliminary Experiments on a Microbial Fuel Cell

Abstract. Experiments were initiated to determine whether microbes using a hydrocarbon as food could generate electrical energy. Experiments with ethane were unsuccessful, but when microbes or glucose oxidase were added to a solution of glucose, electrical output was observed, confirming in part previous observations. As far as we know, no one had previously tested hydrocarbons in this manner, although the several effects of microbial activity on redox potential are well known. Failure of the microbes employed to dehydrogenate ethane in the absence of molecular oxygen is discussed in the light of recently published experimental evidence.

Since biological dehydrogenations take place in the absence of an immediate direct participation of oxygen, it is conceivable that a wire could couple oxygen with the microbial dehydrogenation and hydrogen ionization reactions. The electrons transferred would react at the oxygen electrode to produce hydroxyl ions which could migrate through a semipermeable membrane to react with hydrogen ions, completing the cyclic reaction. It is obvious that the crucial link in the series of reactions is the substitution of a wire for the ordinary electron transport mechanism, at least so far as the final reaction with oxygen is concerned.

A practical experimental system required as a prime consideration the selection of a semipermeable membrane which would separate the "biological electrode" from the oxygen electrode. Such a membrane, according to theory, would allow passage of hydroxyl ions but not free oxygen. The system used was divided into three compartments by common dialysis membranes. The two outer compartments contained the electrodes, while the middle compartment served as a "buffer zone." The dialysis membrane, plus continuous bubbling of O_2-free nitrogen through this zone, effectively prevented any oxygen from reaching the biological electrode compartment.

The volume of each of the three compartments was approximately 400 ml. Platinum sheets (10 by 3 in.) were used in the two outer half-cells as electrodes. Nitrogen and oxygen were bubbled continuously into the biological and oxygen half-cells, respectively. Electrical measurements were performed with the usual laboratory devices.

Nocardia were used in the experimental systems because of their established ability to oxidize hydrocarbons. *Escherichia coli,* a facultatively anaerobic bacterium, and glucose oxidase, an oxygen-requiring enzyme, were selected on the basis of their oxygen requirements. The basal solution employed in all compartments consisted of 1 percent sodium chloride in 0.05*M* phosphate buffer, *p*H 7. When glucose was used as substrate it also was added to all three compartments.

In the first test of the experimental cell the glucose-glucose oxidase system was used. This enzyme catalyzes the aerobic oxidation of glucose to gluconic acid. However, in our system, no reaction occurred when the electrode wire was substituted for oxygen. This could mean either that molecular oxygen is absolutely required for the reaction or that a substitute hydrogen acceptor is required to initiate the reaction in the absence of oxygen. Findings when methylene blue (0.25 mg) was added proved the latter premise to be correct. Open-circuit voltage (electromotive force) increased 80 to 180 mv, and 50 to 100 mv was maintained under a load of 1000 ohms.

Since the glucose-glucose oxidase system failed to react in the absence of oxygen but did react with methylene blue, it was reasoned that a facultative anaerobe, which requires neither of the two in its metabolism, might produce measurable current. Such was the case. When *Escherichia coli* was added to the biological half-cell with glucose as substrate, the open circuit voltage increased from 150 to 625 mv and, under a load of 1000 ohms, 500 mv was maintained for over 1 hour, at which time