gests that staggerer is not identical to, or allelic with, any of these genes.

Linkage tests have been made with five dominant marker genes—*Ra, Os, Mi^{wh}, W^v*, and *T*. The intercross data from the offspring of the fertile staggerer male, plus information from additional breeding tests, provide linkage data for the nonagouti (*a*) and brown (*b*) loci. No close linkage was found with any of these markers, but the recombination with W^v (35.81 ± 9.62), while not significantly different from ½, suggests that more data may reveal a significant linkage. Since *rl* is known to be about 29 units from W^v (*7*), additional tests for allelism between *sg* and *rl* and more linkage tests with marker genes in this linkage group (III) will be made.

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Polyoma and Papilloma Viruses: Do They Have 42 or 92 Subunits?

On the basis of a number of similar properties exhibited by rabbit and human papilloma viruses, polyoma virus, and SV_{40} (vacuolating agent), Melnick (*1*) has suggested grouping these viruses as "papova" viruses types 1 to 4, respectively. The similarities included the appearance of 42 morphological subunits (capsomeres) on the surface of these four viruses. Before these observations of 42 morphological subunits become established criteria for the classification of these viruses, it would seem worthwhile to reconsider the evidence upon which these observations are based.

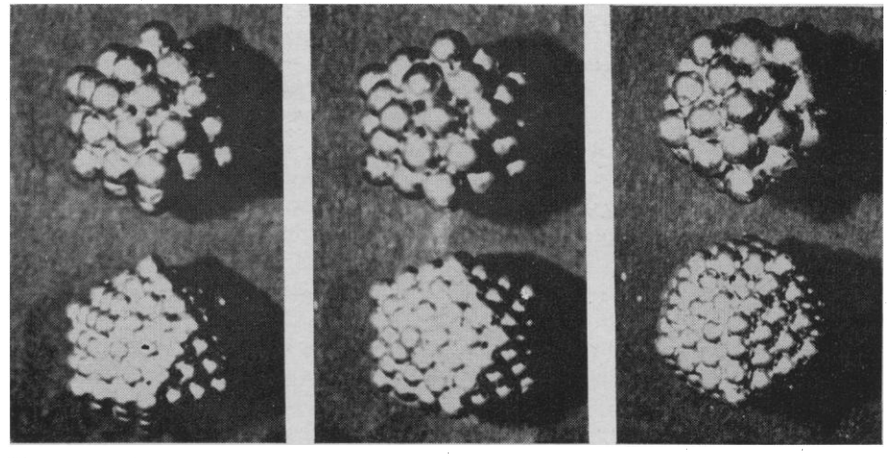

Fig. 1. Icosahedral models constructed of 42 (upper) and 92 (lower) spheres, seen from left to right on 3-, 2-, and 5-fold rotation axes.

Wildy *et al.* (*2*), Howatson (*3*), and Howatson *et al.* (*4*) have interpreted the subunit orientation of polyoma virus and human papilloma virus, respectively, as being of 5 : 3 : 2 symmetry and consisting of 42 morphological subunits, presumably in the configuration of an icosahedron having six shared subunits on each of 20 faces. The studies of Williams *et al.* (*5*) on the Shope papilloma virus indicated that about 30 subunits were visible on about half of the virus particle or "something like 60 knobs on the surface of the entire virus particle." The evidence for 42 subunits on SV_{40}, (*1*) is unconvincing on the basis of published micrographs.

Problems in the interpretation of electron micrographs of viruses negatively stained with phosphotungstic acid (PTA) have been discussed (*5, 6*). On the basis of these discussions and the evidence presented below, it seems at least equally possible that these viruses contain 92 rather than 42 morphological subunits.

In most of the micrographs under consideration there is evidence of obscuring of the virus periphery by a halo of phosphotungstic acid. Williams *et al.* (*5*) clearly demonstrated a decrease in the apparent diameter of the Shope papilloma virus with increasing thickness of the imbedding phosphotungstic acid and an apparent decrease in diameter of PTA-stained particles compared to metal shadowed viruses. Similar obscuring of the edges of tobacco mosaic virus by phosphotungstic acid has been described (*6*). The center-to-center spacing of rods of tobacco mosaic virus in close packing has been well established by x-ray diffraction and microscopy of metal shadowed particles to be 150A. The same spacing is observed between rods imbedded in phosphotungstic acid, but the apparent width of these particles is frequently as small as 120A. It is likely that most negatively stained virus particles are obscured peripherally to a greater or lesser extent. Furthermore, the observation of some 30 subunits on about half of the papilloma virus (*5*) may imply that there are substantially more than 60 subunits on the entire virus surface since one would not expect to resolve clearly those subunits lying in planes which are very nearly perpendicular to the supporting grid.

Models containing 42 and 92 spheres in icosahedral orientation are presented in Fig. 1. They are seen along 3-, 2-, and 5-fold rotation axes, which order corresponds to the decreasing probability of observing viruses of similar shape since they would be in planar, linear, and point contact, respectively, with the grid. The model consisting of 42 subunits would, in all likelihood, present no more than 12 peripheral subunits when viewed on 3- or 2-fold axes and no more than 10 subunits when seen along a 5-fold axis. Most of the virus particles in the studies in question clearly show considerably more than 12 subunits on their periphery. In those particles in which subunits cannot be resolved around the entire periphery, six subunits may be frequently observed on approximately a third of their periphery. This appearance is compatible with the 92 subunit model in which one might see 18 peripheral units

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*).

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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.