Table 1. Effect of antiserum directed against allotypic specificity on phage-neutralizing activity of antiserums obtained from rabbits 8 days after a single injection of bacteriophage T4. Five microliters of antiserum to T4 were added to 1 ml of either normal rabbit serum or antiserum to the indicated allotypic specificity. This mixture was incubated ½ or 1 hour at 37°C and then at 4°C overnight before activity against T4 was assayed.

Serum	Allotypic formula	Antiserum	K_{T4} (min^{-1})	Inhibition (%)
A002	A *a*2*b*4	None*	1.3	
A002	A *a*2*b*4	A2	0.33	75
A002	A *a*2*b*4	A1	1.1	15
A002	A *a*2*b*4	A4	0.26	80
A003	A *a*1*b*4	None*	1.6	
A003	A *a*1*b*4	A1	0.96	40
A003	A *a*1*b*4	A3	1.8	−12
A006	A *a*3*b*4	None*	1.6	
A006	A *a*3*b*4	A3	0.66	59
A006	A *a*3*b*4	A1	1.8	−12
A006	A *a*3*b*4	A1†	1.6	0
A010	A *a*1*b*4.5	None*	1.3‡	
A010	A *a*3*b*4.5	A1	0.1	92
A010	A *a*1*b*4.5	A2	1.1	15
A012	A *a*2*b*4	None*	0.94‡	
A012	A *a*2*b*4	A2	0.1	89
A012	A *a*2*b*4	A1	0.85	10
A013	A *a*1*b*4	None*	0.79‡	
A013	A *a*1*b*4	A1	0.1	87
A013	A *a*1*b*4	A2	0.74	6

* Normal rabbit serum used instead of antiserum. † Normal A1$^+$ serum (5 mμ) was also added to antiserum to A1 to test for indirect effects. ‡ These values are corrected for some T4-neutralizing activity present in the normal serum used for these assays. This correction was about 0.2 K units (0.2 min^{-1}) and was subtracted from each of the final K values of the three serums to yield the values given here.

removed from the 7*S* position as marked by the phage-neutralizing activity of a high-titer antiserum to T4.

Another characteristic of IgG and IgM is the difference in antigenicity of the two immunoglobulins. Antiserum directed against the papain digest fragments I and I′ (or Fab) of IgG will react with both IgM and IgG, whereas antiserum directed against the papain fragment III (Fc) of IgG will react only with IgG (*7*). Furthermore, either antiserum to fragment I or antiserum to fragment III added in excess to high-titer (IgG) phage-neutralizing antiserum will inhibit the neutralizing activity (*15*). The addition of antiserum to fragment I to the first three 8-day antiserums to T4 followed by incubation at 37° and at 4°C resulted in the loss of 56 to 90 percent of the T4-neutralizing activity; antiserum to fragment III inhibited these antiserums to T4 less than 10 percent in two cases and 20 percent in the third case, and these inhibitions with antiserum to fragment III could easily be explained by nonspecific effects in view of the large amount of precipitation.

Thus antiserum directed against the *a*-group specificity of early antiserums against bacteriophage inhibits phage-neutralizing activity. This inhibition is apparently due to a direct action on IgM—at least that particular IgM represented by 19*S* antibodies to T4. Also, the phage-neutralizing activity of such 8-day antiserum was mainly IgM by all criteria tested; the amount of 7*S* antibody (IgG) which could be present would not be enough to modify the conclusions that the foregoing effects were due to action on IgM.

The chemical nature of the *a*-group allotypic specificities is not known at present; the specificities could be due either to variations in the protein primary structure or to variations in nonprotein moieties—perhaps carbohydrate—added to the polypeptide chains of either IgG or IgM protein. But if the *a*-group specificities are due to variations in the primary structure of the polypeptide chains, the suggestion by Todd (*1*) and again by Cohen and Porter (*6*) that the primary heavy chain actually is at least two polypeptide chains, with at least one portion common between IgG and IgM, must be very seriously considered. It should be pointed out that the two polypeptide chains might fuse to a single chain after their independent synthesis. Again, a mechanism such as suggested by Smithies *et al.* (*16*) would allow as another possibility the formation of a single polypeptide chain with its primary structure controlled by nonadjacent genetic elements. It should be possible before too long to choose from these or other suggestions to form a clearer concept of the biosynthesis of the immunoglobulins.

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17. Supported in part by a NSF summer fellowship for graduate teaching assistants to RJF.

26 July 1965

Rotational Symmetry in Foot-and-Mouth Disease Virus and Models

Abstract. *With small, animal viruses for which electron-microscope images show little penetration by negative stain, the rotation technique for structure determination is useful. Foot-and-mouth disease virus resembles a 32-unit model when rotational images of virus and models simulating virus in negative stain are compared.*

The smaller animal viruses do not show the same resolution of morphological subunits in negatively stained preparations for electron microscopy as some other viruses of the same general size do. For example, foot-and-mouth disease virus (FMDV) and poliomyelitis virus (*1*) are not as easily penetrated by phosphotungstic acid as turnip yellow mosaic virus (*2*) and ϕX174 virus (*3*) are. To determine the probable arrangement of morphological subunits in FMDV, the rotation technique of Markham (*4*) was applied to images of highly purified virus as well as to models constructed to simulate the condition of virus in negative stain.

Foot-and-mouth disease virus, strain A119, was produced in tissue cultures of bovine kidney and purified (*5*). Virus was deposited by loop-drop on formvar films lightly coated with carbon. The grids were stained with 1 percent potassium phosphotungstate (*6*) and examined in an electron microscope (RCA-EMU-3G) with a double condenser. Selected micrographs of individual virus particles (Fig. 1) indicate that morphological units are visi-

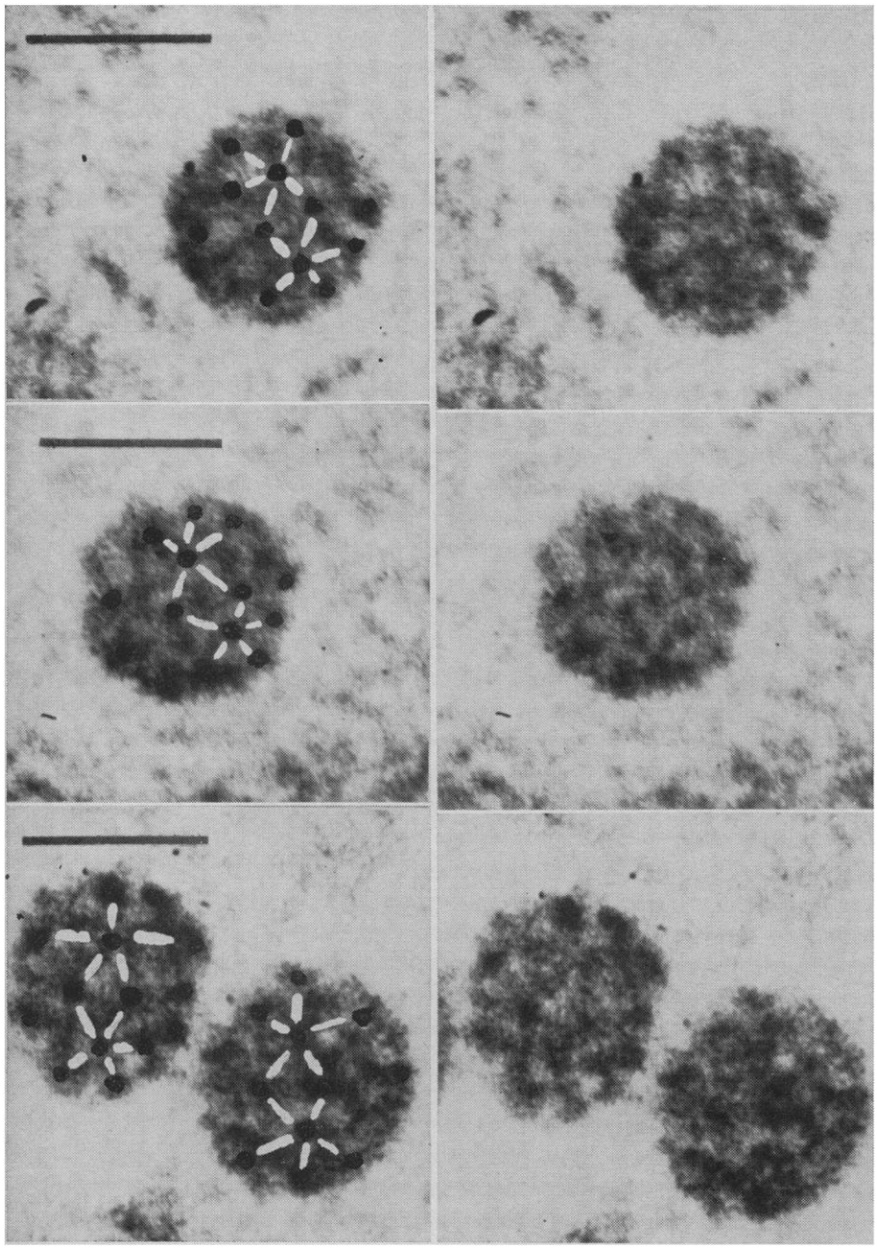

Fig. 1. FMDV, strain A119, virus particles at high magnification, printed in reverse contrast after negative staining. In the left column particles are marked with possible centers of fivefold symmetry; in the right column the same particles are unmarked. Magnification bar, 250 Å.

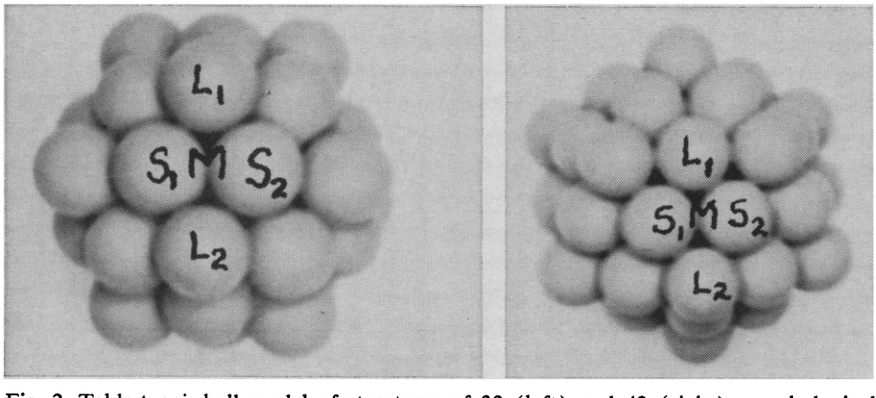

Fig. 2. Table-tennis-ball model of structures of 32 (left) and 42 (right) morphological units, marked at the center of the rhombus (M), at the ends of the short diagonal (S_1 and S_2), and at the ends of the long diagonal (L_1 and L_2).

ble and that 32 or 42 might form the virus.

Models with 32 or 42 morphological units were constructed of table-tennis balls and small spheres separated by sticks. The 32 units were arrayed as a rhombic tricontahedron, and the 42 were arranged as an icosahedron. The models (Fig. 2) illustrate the features common to the two, and S_1, S_2, M, L_1, and L_2 are the ends of the short diagonal, center, and ends of the long diagonal of the central rhombus (*1*). These serve as the centers of rotation in the Markham (*4*) technique and also indicate that rotations might be used to distinguish between them. In the rhombic tricontahedron, morphological units at either end of the short diagonal are surrounded by six others, whereas those at the ends of the long diagonal are surrounded by five. In the icosahedron the ends of the short diagonal are surrounded by six units, but on the long diagonal one end is surrounded by five and the other end by six. This inequality occurs because the long diagonal extends from a vertex to the center of an edge of a face of the icosahedron. Therefore, six-step rotations about the ends of short diagonal and, depending on the model, either five- or six-step rotations about the ends of the long diagonal should lead to reinforcement.

The models in Fig. 2, however, do not simulate the structure of a negatively stained virus particle as accurately as does the expanded model with separated units, shown in Fig. 3, row 1, columns A and D. These simulate virus particles as seen in phosphotungstic acid preparation with the density contributed by all the particles. Photographic negatives of the models were also opaqued so that only units on the upper half remained (Fig. 3, row 1, columns B and D). Such models would correspond to electron-microscope images of viruses embedded in phosphotungstic acid when some units were obscured. The image in Fig. 3 (row 1, column C) is an FMDV particle showing capsomeres enclosing a central rhombus. Comparison of several views of open and opaque models with stained virus particles reveals that both upper and lower structural units may be visible in the micrographs.

Image reinforcements were made of five- and six-step rotations about corresponding points in the model and virus negatives; Fig. 3, row 2, shows a six-step rotation about the center of

the rhombus (M); rows 3 and 4 show six-step rotations at each end of the short diagonal (S_1 and S_2 positions); rows 5 and 6 are five-step rotations about the ends of the long diagonal (L_1 and L_2); and rows 7 and 8 are six-step rotations about the same centers. The enlargements in the center column of Fig. 3 are rotations of FMDV-particle images selected to provide reinforcements. Not every virus particle may be used successfully for all rotations.

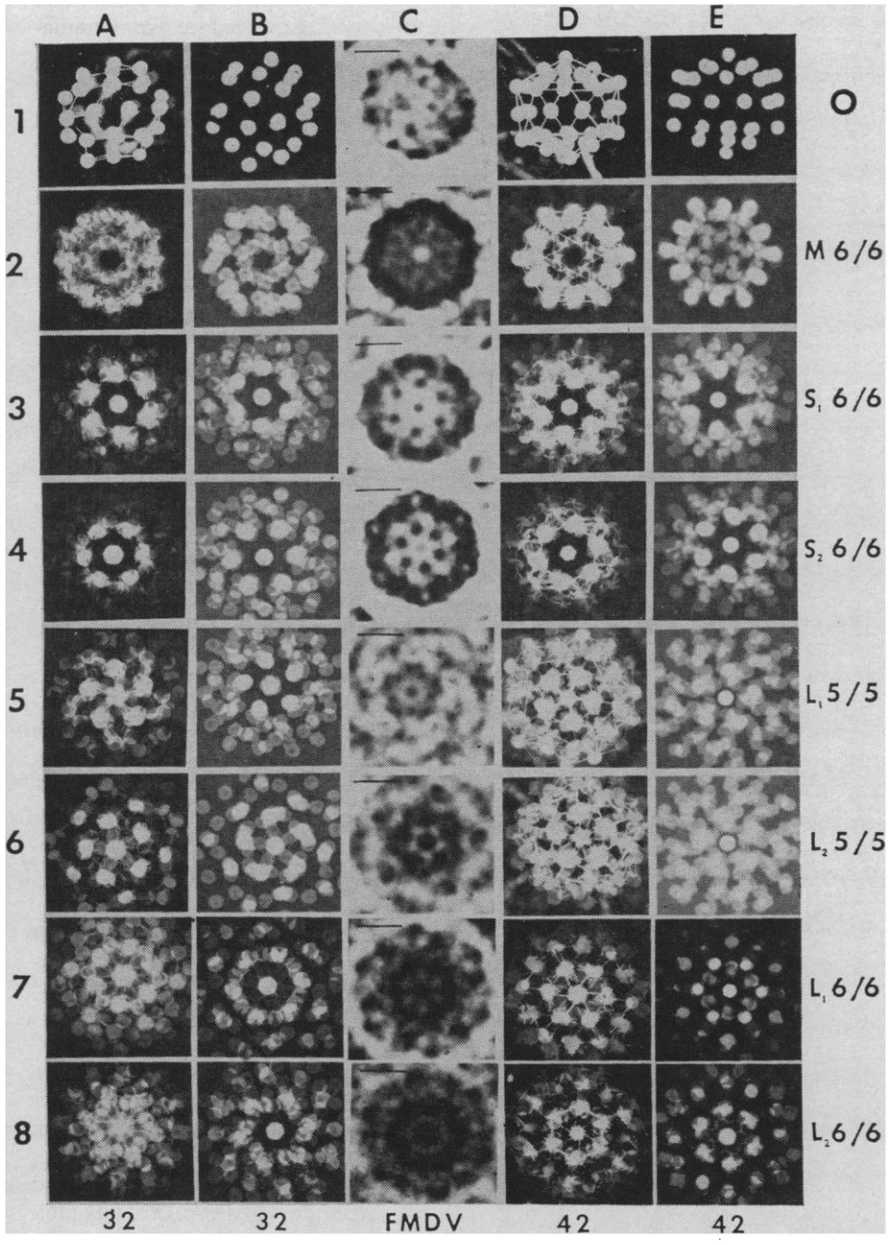

Fig. 3. Column A, images of a 32-unit rhombic tricontahedron with all particles and connectors visible; column B, images of a 32-unit model in which particles on the lower surfaces and connectors have been opaqued; column C, images of FMDV, strain A119, virus particles, selected to show salient features (magnification bar, 100 Å); column D, images of a 42-unit icosahedron in which all particles and connectors are visible; column E, images of a 42-unit model in which particles on the lower surfaces and connectors have been opaqued. Row 1, images without rotation (O); row 2, images after six-step rotation about center (M) of the prominent rhombus ($M6/6$); row 3, images after six-step rotation about the unit at one end of the short diagonal (S_1) of the rhombus (S_1 6/6); row 4, images after six-step rotation about the unit at the other end of the short diagonal (S_2) of the rhombus (S_2 6/6); row 5, images after five-step rotation about the unit at one end of the long diagonal (L_1) of the rhombus (L_1 5/5); row 6, images after five-step rotation about the unit at the other end of the long diagonal (L_2) of the rhombus (L_2 5/5); row 7, images after six-step rotation about the L_1 position (L_1 6/6); row 8, images after six-step rotation about the L_2 position (L_2 6/6). For greatest clarity of reinforcement model images were printed as white on black background whereas the reverse was done with the virus micrographs.

The rotation technique, even when applied to the models in which the structural units are clearly defined, does not make many clear distinctions between structures of 32 and 42 units. In Fig. 3, row 2, rotation about the center of the rhombus gives good reinforcement in the outer ring with a 42-unit model, but not with a 32-unit model or with the virus in column C. In Fig. 3, row 6, the correspondence between the patterns of reinforcement is greater between the virus in column C and the 32-unit models in columns A and B than with the 42-unit models. The same may be said for rows 7 and 8.

On the basis of the rotation technique and comparison of virus images and models, FMDV would be classified as a 32-unit virus. Although this method may not give the detailed information that can be obtained when negative stain and models are applied to the analysis of larger viruses (*7*), it does emphasize the difficulty of interpretation with small viruses. The rotation technique does not unequivocally distinguish between a 32- and 42-unit model in any single rotation pattern. When the technique is applied to structures as likely to be distorted as virus particles in phosphotungstic acid are, the possibility of accurate structure determination is reduced. In a similar study Chambers *et al.* (*8*) chose a 42-unit morphological structure for the Gladiolus virus, which is similar in size to FMDV.

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9. We thank S. J. Pyne and C. E. Bennett for assistance in virus purification and preparation of electron-microscope specimens.

6 August 1965