Brief Note

The Packing Structure of Crystalline RI-APC Virus.* By Barbara W. Low and Peter R. Pinnock. (*From the Department of Biological Chemistry, Harvard Medical School, Boston.*)‡

In a paper in the preceding issue of this *Journal* Morgan, Howe, Rose, and Moore (1) have described their studies of electron micrographs of sections of tissue infected with RI-APC viruses. The virus particle aggregates appear most frequently as well ordered two-dimensional arrays. Studies of serial sections have shown that these two-dimensional nets are planes through three-dimensional virus crystals.

We have had the opportunity to examine these electron micrographs. Our study has suggested that the viral particles are packed in a cubic body–centered lattice. The determination of the probable crystal lattice structure of a virus from section micrographs cannot, for several reasons, be as certain as a determination using replica or pseudo-replica techniques (2). In the electron micrograph of a replica, the simultaneous view of two non-parallel faces which intersect along an edge may provide powerful visual evidence concerning the intermolecular packing in three dimensions. The cutting of sections, as in this study, of thickness less than the diameter of a single particle will in general produce marked distortion either in particle shape or in interparticle alignment, perhaps in both. The extent of the distortion will be variable since it will depend on many different factors. These will include the nature of the plane of section and the orientation of the cutting edge within the plane. In general the most marked changes in the virus shape will occur when particle distortion is the only means of relieving the compression. The most marked changes in the shape and size of the lattice unit will occur when rows of virus molecules can relieve the compression by moving with respect to each other. The extent of the distortion has been observed directly in one instance by Morgan and his coworkers. A thick section of tissue was examined in the light microscope and a photomicrograph was taken of a single diamond-shaped crystal embedded in a cell. The same crystal was then examined electron microscopically in a contiguous thin section. The angles measured in the original photograph were approximately 100° and 80°. In the thin section electron micrograph, the angles were approximately 135° and 45° respectively.

Morgan and his coworkers have shown that the particle distortion appears as a lengthening in a direction parallel to the knife edge, roughly proportional to the shortening in the direction perpendicular to it. From their studies they have concluded that the virus molecules are spherical particles of diameter between 600 and 650 A. They have also shown (3) that central sections of maximum volume

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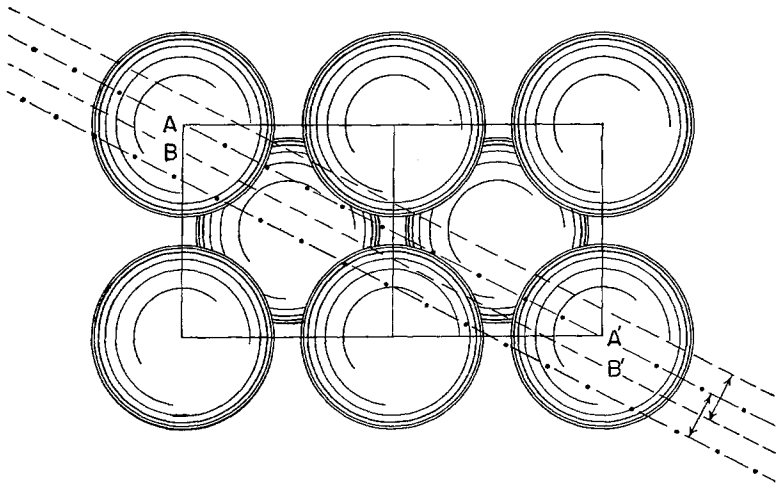
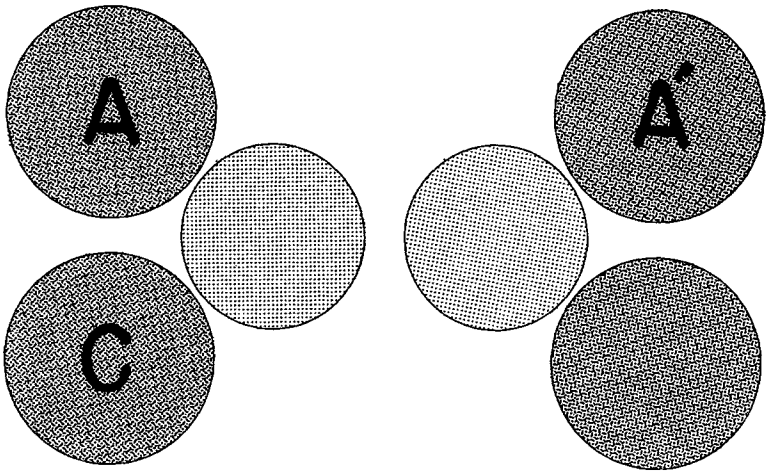


TEXT-FIG. 1. Cubic body-centered structure projected on the c plane showing the traces of two sections both parallel to the (120) plane. The width of the section corresponds to a 200 A thickness for a particle of 650 A diameter.



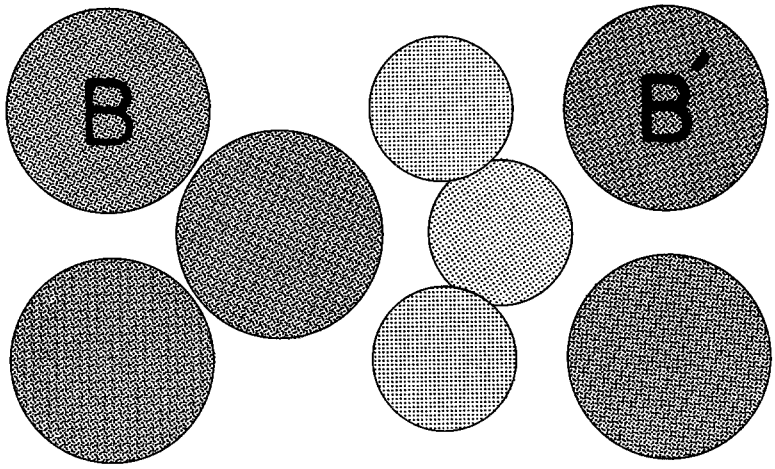
TEXT-FIG. 2. Appearance of plane through center ($A - A'$) of 200 A thick section. The circles correspond directly to the areas of the particles in this plane. The variation in tone corresponds approximately to the variation in volume of the whole section of each particle.

through viral particles will appear as more dense, sharply defined areas on a micrograph than will non-central sections (of smaller volume and average cross-section area). Dark gray areas on the micrograph will thus in general correspond to central sections through the virus particles and lighter gray areas will correspond to regions of smaller volume. (The heavy unrelieved black shading of certain particles which appear scattered at random throughout the

section has been associated by Morgan *et al.* with quantitative differences between the virus particles themselves rather than with qualitative differences of particle section volume.)

There are three close packed arrangements of spheres. In both (1) cubic closest packings and (2) hexagonal close packing each sphere is surrounded by twelve nearest neighbors with six nearest neighbors in each close packed layer. In a cubic body-centered lattice each sphere has eight nearest neighbors as shown in Text-fig. 1 of the paper by Morgan and his colleagues (1).

We have considered all three close-packed structures, particularly at first in relation to the probable packing in the section shown in Fig. 11 (Morgan *et al.* (1)). This appeared to correspond rather closely to the distribution which might be expected in a section of the (120) plane through a body-centered cubic lattice. Text-fig. 1 shows the trace of two sections 200 A thick parallel to the (120) plane of a structure with particle diameter 650 A. The thickness of the section is indicated by the extent of the double headed arrows. The section centered about A–A' will appear as shown in Text-fig. 2, and the section centered about B–B' will appear as shown in Text-fig. 3. It has been shown that changes in the diameter of particle sections do not in general appear directly on the micrograph; they may be inferred from the changes in density and sharpness of the image. In Text-figs. 2 and 3 we have tried to show the relationship between the observed changes in blackening and the assumed variation in section areas.



TEST-FIG. 3. Appearance of plane through center (B — B') of a 200 A thick section. The circles correspond directly to the areas of the particles in this plane. The variation in tone corresponds approximately to the variation in volume of the whole section of each particle.

Packing regions can be found in the micrograph which appear to correspond either to Text-fig. 2 or 3. If the section is not cut absolutely parallel to (120) changes will occur over the whole region of the micrograph. These changes may be further enhanced by two other factors: (1) there may be faults in the structure and (2) the lattice may be

only pseudocubic because of particle asymmetry.

The ratio of the sides of this unit AA'/AC should be $\sqrt{5:1}$. For a particle of diameter 650 A their absolute lengths should be 1671 A and 750 A respectively. Measurements of Fig. 11 (1) give several values for the dimension for this unit. These are within the range $AA' = 1680 \pm 40$ A, $AC = 880 \pm 25$ A. The ratio of the sides is therefore somewhat less than $\sqrt{5:1}$. The side AC appears rather too long. If the particle diameter were longer than 650 A compression of the longer axis could be invoked to explain the discrepancy. The plane of section of Fig. 11 might, however, be the (121) plane. The ratio of the lengths of the sides of the unit would then be $\sqrt{5} : \sqrt{2}$ and the lengths of these sides would be 1671 A and 1060 A respectively. The unit would not be rectangular although the particle packing would be similar to that in that (120) plane. Angular distortion combined with some compression of the shorter axis would give a reasonable fit. In general it is somewhat easier to explain a shortening of the unit dimensions than to account for apparent lengthening.

For several reasons we have presented a detailed discussion of the identification of the section in Fig. 11 (1). First, an acceptable packing model must be able to provide a reasonable explanation for the parallel zones of dense and light particles which appear in some micrographs. Second, this example illustrates our general approach to the problem of identifying the packing, and third, it shows that the validity of this interpretation rests upon the permissible degree of variation between the dimensions of the proposed structure and the dimensions measured from the electron micrographs.

Our identification of the packing is based therefore less on the correspondence between any one micrograph and the model structure than on an extensive study of all the electron micrographs published by Morgan *et al.*, and of several other unpublished photographs.

The two alternative closest packed arrangements can be eliminated; equally a primitive non-centered packing arrangement can be ruled out. On the other hand it has been possible to identify tentatively the packing in the planes of sections in all these micrographs on the basis of a cubic body-centered lattice. In most of the micrographs it was necessary to assume some particle distortion or particle realignment in order to account for the packing observed.

One further comment may be made. Our experience in interpreting electron micrographs is extremely limited. In interpreting the packing we have therefore accepted the premise that these viral particles are spherical and that their diameter is within the range 600 to 650 A. We have, however, the impression, from a detailed study of the particle packing in many micrographs, that the viral particles are not quite spherical, although both major and minor axes may have lengths within the range 625 ± 25 A.

References

1. Morgan, C., Howe, C., Rose, H. M., and Moore, D. H., *J. Biophysic. and Biochem Cytol.*, 1956, **2**, 351.
2. Wyckoff, R. W. G., Electron Microscopy, New York, Interscience Publishers, 1949. Williams, R. C., *Advances Virus Research*, 1954, **2**, 183.
3. Morgan, C., Rose, H. M., and Moore, D. H., *J. Exp. Med.*, 1956, **104**, 171.