The 10× Sharples concentrate had 1800 CCA units while the final product had 22,000 CCA units per milligram of protein. Similar results are obtained with formalin-inactivated or with infectious virus.

In contrast to the relatively impure starting material used in these studies, the highly purified virus preparations passed through Millipore filters (0.45 μ) without loss of virus.

The purified, formalin-inactivated virus, given as a single immunizing inoculation, protected mice challenged intranasally to the same extent as impure virus did when given at the same virus concentration. The mice protected by purified egg-grown virus developed neutralizing antibodies against influenza virus adapted in tissue culture and against virus adapted in mice. The pure virus, in contrast to the starting material, was nonpyrogenic in rabbits. To date, these properties, as well as hemagglutinin (standard CCA test) have been stable for at least 8 months at 4°C. Therefore, this purification process is not detrimental to these biological characteristics of the virus.

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28 March 1966

Pentagonal Aggregation of Virus Particles

Abstract. *Virus aggregates with a unique fivefold axis have been observed in the electron microscope.*

Bagley (*1*) has described a dense structure with fivefold symmetry (Fig. 1, left) which results from the continued packing of hard spheres on a pentagonal bipyramidal nucleus. The growth of such a nucleus was proposed as a possible mechanism to account for a number of observations of apparent fivefold symmetry in crystals (*2*). These had earlier been explained as quintuple twins about a common axis (110) of face-centered cubic individuals, with lattice strain or imperfections making up the angular deficit of 7°20′.

During the preparation of bacteriophage f1 a contaminant phage fraction was isolated by differential centrifugation. Because our laboratory is involved in the large-scale growth of R17, we believe it to be the contaminant. Negatively stained electron-microscope preparations of both the contaminant and R17 phages appear identical. In an electron micrograph of an area in which both the contaminant virus concentration and the specimen thickness were so great as to approach the limits of electron transmission, we were surprised to find two pentagonal groups of viruses (Fig. 2) which we had not seen on the electron-microscope viewing screen. Although we have been unsuccessful in finding further examples of this type of virus packing, the chance observation

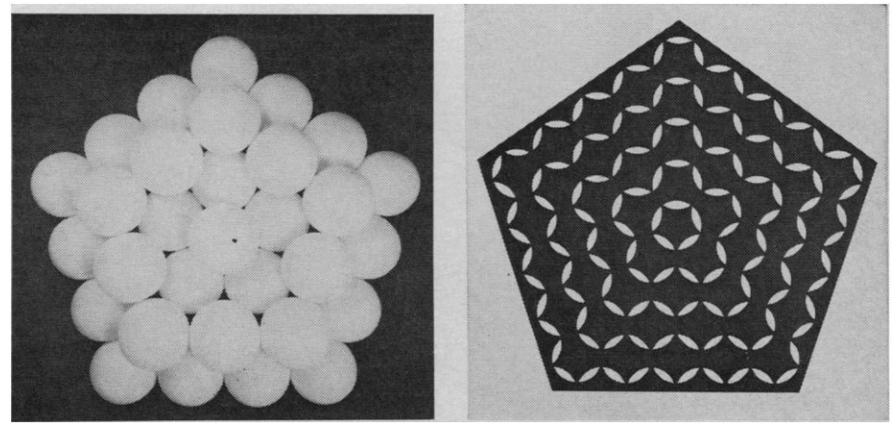

Fig. 1. (Left) Model of the packing system proposed by Bagley (*1*). (Right) Staining pattern predicted if stain fills the gaps between virus particles arranged at the left.

of the first two groups indicates that they occur in regions not easily inspected in the electron microscope.

The stain appears to lie in the interstices between the virus particles, so that only those regions where the particles overlap are transparent to the electrons. The idealized staining pattern expected from Bagley's model is shown in Fig. 1, right. The variation in length of the pentagonal sides in Fig. 2 (of the order of 5 percent) may be due to uneven dehydration during specimen preparation or the tilt of the plane of the pentagon face with respect to the photographic film. Nevertheless, the numbers of individual particles on each side can be counted and are clearly in agreement with Bagley's model. In the pentagonal sectors in which the unit cell is body-centered-orthorhombic, the ratio of the cell dimensions predicted by Bagley is b/a = cotangent 36° = 1.376. We measure $b/a = 1.39 \pm 0.05$. The extent of the pentagonal aggregates observed is about 2000 Å (each virus particle is about 200 Å in diameter). The thickness cannot be measured, but is at least two layers.

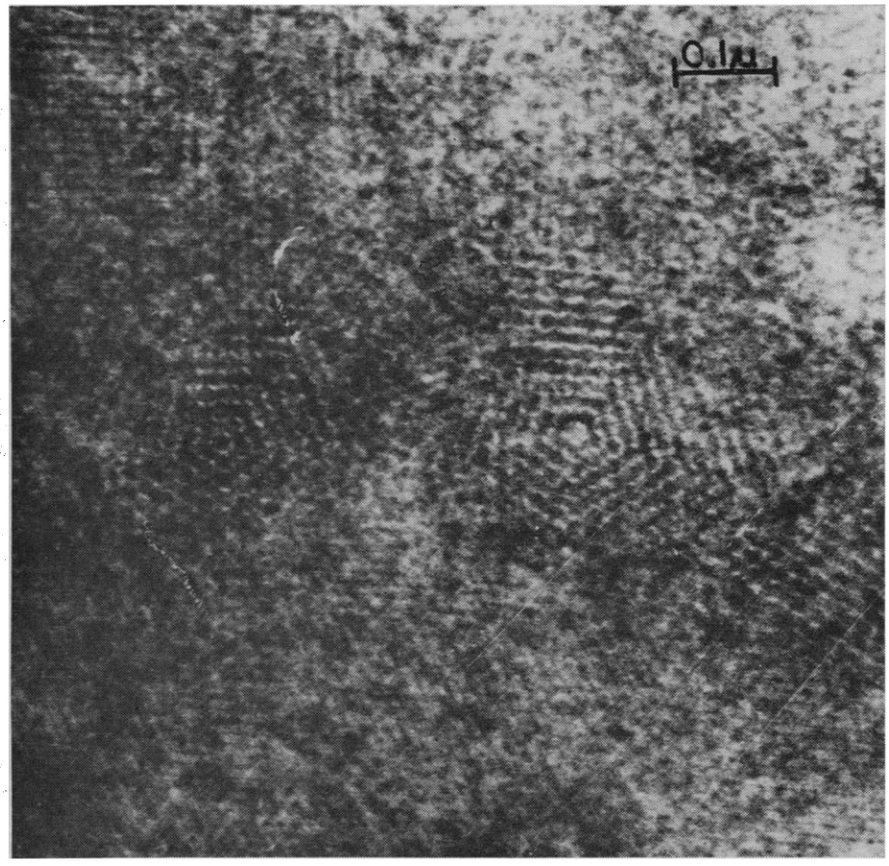

Fig. 2. Electron micrograph of bacteriophage negatively stained with 2 percent phosphotungstic acid, *p*H 5.5.

Although the pentagonal symmetry is obvious by inspection, it is emphasized by image rotation superpositions of the type pioneered by Markham (*3*). Five exposures of one-fifth normal time were made of each pentagonal group, the object being rotated 72° about the symmetry axis after each exposure (Fig. 3).

Fivefold symmetry axes are already present as elements of the point group symmetry (532) of the outer protein shell of all small "spherical" virus particles which have been studied in detail, and, although no observations have yet been reported, it is unlikely that R17 is an exception to this general principle (*4*). Therefore, a single virus particle may be a suitable nucleus for fivefold aggregation of other virus particles, and it is possible to see how the pentagonal bipyramidal nucleus suggested by Bagley might form. In the body-centered orthorhombic unit cell the best packing is achieved if a twofold axis of each virus particle is perpendicular to the photograph (that is, rotated relative to the center particle). The present electron micrographs are not sufficiently detailed, mainly because of the thickness of the specimen, to determine the orientation of the individual virus particles.

Macroscopic virus aggregates of this form have not been reported, although,

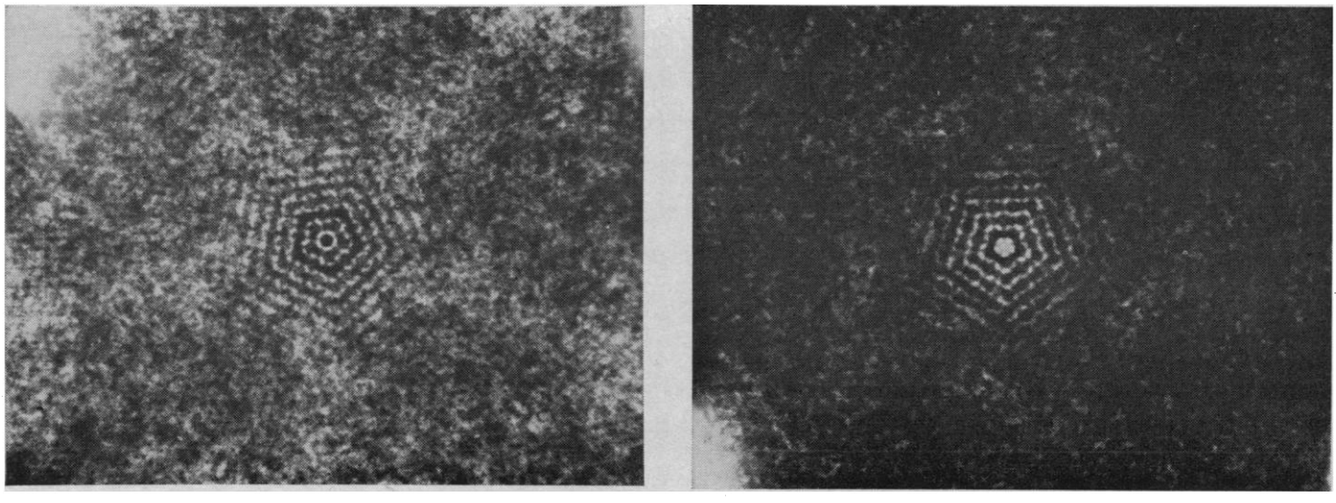

Fig. 3. Rotation superpositions of each of the two fivefold groups in Fig. 2.

as Bagley points out, an extended almost close-packed space-filling structure can be generated. It is a moot point whether or not such a structure could be considered a crystal.

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Quantum Yield of Oxygen Evolution and the Emerson Enhancement Effect in Deuterated Chlorella

Abstract. *The maximum quantum yield of oxygen evolution in deuterated chlorella is found to be 0.075, while normal chlorella showed, in parallel experiments, a value of 0.10. Deuterated* Chlorella vulgaris *showed a decline in the quantum yield of oxygen evolution ("red drop") beginning at 680 millimicrons and a clear Emerson enhancement effect qualitatively similar to that obtained in normal chlorella cells. However, the ratio of quantum yield at 680 to that at 710 millimicrons was about 1.5 times higher in normal than in deuterated chlorella cells. Action spectra of the Emerson enhancement effect in deuterated chlorella also are qualitatively similar to those of normal cells.*

It is now widely accepted that photosynthesis requires two light reactions (*1*) and that, because of this, normal chlorella cells show the so-called Emerson enhancement phenomenon (*2*). The importance of deuterated organisms in biological research has been recently reviewed by Katz and Crespi (*3*). Whether the two light reactions are necessary for deuterated chlorella was uncertain, because Emerson (*4*) was unable to observe the enhancement effect in several samples of partially deuterated chlorella. We investigated the action spectrum of photosynthesis in completely (99.5 percent) deuterated *Chlorella vulgaris* and observed the usual decline in quantum yield of photosynthesis beyond 680 mμ ("red drop") and the Emerson enhancement effect.

Deuterated *Chlorella vulgaris* (obtained from J. J. Katz and H. L. Crespi) was cultured in inorganic salt medium (*5*), and the cells, harvested after 4 to 7 days' growth, were suspended in deuterated Warburg's buffer No. 9 (15 parts 0.1*M* K_2CO_3 and 85 parts 0.1*M* $NaHCO_3$ in D_2O) in a volume concentration of 0.4 to 0.5 percent. Measurements of O_2 evolution were made with a differential manometer (*6*). The manometric vessels, submerged in a constant-temperature bath (9.6° ± 0.1°C), were continuously shaken with a frequency of 200 oscillations per minute. Light (bandwith of beam, 5 to 10 mμ) from the large Emerson-Lewis monochromator (*7*) entered the reaction vessel from the bottom; supplementary (or background) light also entered the vessel from the bottom. Supplementary beams were produced by filtering white light with Baird-Atomic interference filters (60 to 80 percent transmission at their peaks) which had half-bandwidths of 8 mμ. Both light sources were tungsten lamps.

Quantum yield determinations required measurements of the number of absorbed quanta. The number of incident quanta was measured by a large-surface bolometer calibrated against a radiation standard from the U.S. Bureau of Standards. Percent absorption at different wavelengths was measured by two instruments: (i) a Bausch and Lomb (Spectronic 505) spectrophotometer, equipped with an integrating sphere and (ii) an integrating sphere spectrophotometer constructed by Cederstrand (*8*) in our laboratory. Aminco cuvettes were used in the Bausch and Lomb instrument, while in the other instrument manometer vessels were used directly. Measurements with the Cederstrand instrument were considered more reliable and were used for quantum yield calculations, but values obtained with the Bausch and Lomb instrument deviated from the former by less than 5 percent. All measurements (unless otherwise specified) were made in the linear part of photosynthesis versus light-intensity curve.

Table 1. Rates of O_2 evolution and O_2 uptake in deuterated and normal cells of *Chlorella vulgaris*. All results are expressed in millimicromoles per liter per hour.

Rate of O_2 evolution*		Rate of O_2 uptake†	
Deuterated cells	Normal cells	Deuterated cells	Normal cells
Weak white light (5%)			
496	625	315	289
Strong white light (100%; saturating)‡			
868	3022	360§	431§

* Rate of O_2 evolution was corrected for differences in percent absorption in the two samples. † Respiration rate (for same volume of cells) was calculated by taking the average of rate of O_2 uptake before and after illumination. ‡ Saturation of O_2 evolution checked by changing light intensity by placing 60-, 70-, and 80-percent transmitting neutral density filters and finding a constant rate of photosynthesis. § Respiration rate for chlorella was higher because the higher rate of photosynthesis with 100-percent light caused an increased O_2 uptake.

On the basis of a minimum of three experiments performed on different cultures for each of the phenomena reported, the following conclusions were reached for deuterated cells of *Chlorella vulgaris*.

In deuterated chlorella the rate of O_2 evolution measured at saturating light intensities was reduced by a factor of 3 (Table 1); when measured in weak light intensity the rate was reduced only slightly (about 20 percent). (Several other experiments gave qualitatively similar results.) The findings of Craig and Trelease (*9*) are thus partly confirmed—they had concluded that deuteration causes a decrease in the rate of rate-limiting chemical reactions but no difference in the rate of photochemical reactions. Rates of O_2 uptake were quite comparable in both deuterated and normal cells, except that after exposure to strong light normal chlorella showed a larger increase in rate of uptake than deuterated cells did (Table 1).

There is a definite decline ["red drop" (*7*)] in the quantum yield of O_2 evolution by deuterated cells at wavelengths beyond 680 mμ (Fig. 1). The maximum observed absolute quantum yield in deuterated chlorella was 0.075; chlorella grown in ordinary water under comparable conditions gave a maximum yield of 0.10. (We do not claim that higher values cannot be obtained under other conditions.) The