Weil bei gleichem chemischem Umsatz der O₂-Druck in dem kleinen Gefäß schneller steigt als in dem großen Gefäß.

Anmerkung. Da Photosynthese = O₂-Entwicklung im Licht + O₂-Verbrauch der Atmung ist, so war — bei einfacher Überkompensation der Atmung — der korrekte Wert der Photosynthese

\[ 2 \cdot \frac{dp}{dt} \cdot kO₂. \]

Da sich aber der Faktor 2 überall heraushebt, wurde er fortgelassen.


The Structure of Tobacco Mosaic Virus as revealed in Ultrathin Sections by Electron Microscopy

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Considerable information is already available on the fine structure of the tobacco mosaic virus based on X-ray diffraction studies of oriented preparations, which can be correlated with the results of biochemical studies. However, electron microscopy observation performed hitherto on tobacco mosaic preparations dried on the specimen films and treated with various reagents or shadow-cast with heavy metals, have revealed only certain details of the internal structure of the rod-shaped particles.

It appeared therefore of interest to apply the techniques of staining, embedding and ultrathin sectioning to a study of the fine structure of tobacco mosaic virus particle by high resolution electron microscopy. These techniques have been successfully em-

2 H. Fernández-Morán, Ind. Diamond Rev. 16, 128 [1956].

We have used the electron microscope with a Hitachi HU 11A. The preparations were examined at an accelerating voltage of 100 kv. The pictures were taken with the Jena H. 3 objective lens.


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Fig. 1. Ultrathin longitudinal section of tobacco mosaic virus particles stained with 1% uranyl acetate and embedded in methacrylate showing internal fine structure. 250 000 X.

Fig. 2 a, b. Longitudinal sections of TMV particles stained with 1% uranyl acetate showing dense para-axial double lines with indications of fine structure. (a) 600 000 X, (b) 460 000 X.

Fig. 3. TMV particle dried on specimen film and stained with 1% uranyl acetate showing distinctly outlined central channel. 400 000 X.
Fig. 4 a, b, c. Transverse ultrathin section of TMV particles from oriented preparation stained with 1% uranyl acetate showing light central area surrounded by dense annular structure with indications of a rosette-type pattern (arrows) (a) 650,000 ×, (b) 670,000 ×, (c) 820,000 ×.

Fig. 5. Longitudinal section of TMV particles stained with ammonium vanadate showing light axial area bounded by dense outer layers with indications of fine structure. 250,000 ×.

Fig. 6. Longitudinal section of bundle of TMV particles stained with ammonium vanadate showing system of dense double bands alternating with lighter spaces. 330,000 ×.
ployed in the investigation of the macromolecular structure of various types of virus particles, and are particularly revealing when dealing with paracrystalline systems. By staining and ultrathin sectioning of oriented TMV preparation it has now been possible to observe certain regularly occurring features of the internal structure of the virus particles which seem to be in general agreement with the model derived from X-ray diffraction studies.

Preparation techniques

Oriented samples were prepared by sucking the stiff gel of fresh tobacco mosaic virus into plastic or glass capillaries (0.2 mm internal diameter). Specimens with random orientation of the particles were also obtained from droplets of the gel collected at the ends of plastic or wood fibers. Short segments of the samples were stained with cold (4°C) aqueous or alcoholic 1 per cent solutions of uranyl acetate, or lanthanum nitrate, phosphotungstic acid, phosphomolybdic acid, and ammonium vanadate. Embedding in prepolymerized butylmethacrylate was carried out after dehydration by passage through a graded series of ethanol. Alternatively, gelatin embedding was tried, thus avoiding the effects of ethanol treatment. The blocks containing the short segments of capillary tubing with the fixed TMV specimens were readily oriented for sectioning in the desired plane. Ultrathin serial sections of 100–200 Å were prepared with a Morán ultramicrotome using a diamond knife. Confirming previous investigations, gelatin embedding was found to be an essential technical requirement in interpreting structural relationships at the macromolecular level. The sections were mounted on fenestrated Formvar films with regularly distributed pores of 200 to 2000 Å, which are particularly suitable for high resolution studies since the edges of the minute holes facilitate rapid focusing, and many areas of the sections can be examined free from the supporting substrate. Control specimens were prepared by applying the same type of stains to suspensions of TMV dried on the specimen screens.

An RCA EMU 3 B and a Siemens ELMISKOP 1 connected to a specially regulated power supply were used in this study. The micrographs were taken at electron optical magnifications of 20,000 X to 42,000 X. The preliminary observations described here are based on evaluation of 700 plates in which an average resolution of 10 to 20 Å was consistently achieved.

Results

The appearance of the stained and embedded TMV particles in thin sections is very similar to the well-known images of TMV specimens dried directly on the films, as regards size and general configuration. The individual particles are about 3000 Å long and 150–160 Å wide, but association of the particles into ribbon-like structures approximately 150 Å wide was more commonly encountered (Fig. 1). Closely packed bundles of particles in regular array are seen sectioned longitudinally and transversely in the oriented preparations (Figs. 2, 4). The following details of internal structure can be discerned in the particles according to the staining procedure employed.

Uranyl acetate and lanthanum nitrate staining

In the majority of the longitudinal and oblique sections through TMV particles stained with uranyl acetate (Fig. 1*, 2) two dense parallel lines can be seen running along the center of each particle. The dense lines have an average width of 20 Å, with indications of a granular structure, and are separated from each other by a light space of 25 to 30 Å (Fig. 2). The outer layer of the particle has a lower density, but the margins stand out distinctly against the background of the embedding medium. This characteristic para-axial double line system found in sectioned specimens stained with uranyl acetate or lanthanum nitrate, is different from the central dense line first observed by in stained TMV particles. A uniform central line about 20–30 Å in diameter can be regularly observed in the virus particles dried on the specimen film and stained with uranyl acetate (Fig. 3). However, in agreement with it probably represents the internal axial channel of the particle outlined by deposition of the stain. Therefore, by virtue of its dimensions and axial location this single central line would correspond more closely to the light intermediate space between the two internal dense lines of the virus particle observed in sectioned preparations.

References

5. J. D. Watson, Biochim. biophysica Acta [Amsterdam] 13, 10 [1954].
7. Figs. 1–6 s. table p. 68 a u. b.
In transverse sections the most notable feature is the distinct appearance of a clear circular area, with a diameter of 25–30 Å, in the center of each particle (Figs. 4 a, b, c). This central opening or hole is surrounded by a concentric ring of dense granules, 15 to 20 Å in diameter, with indications of a regular arrangement in a rosette-type of pattern. In suitably oriented oblique sections through the virus particles the double para-axial lines which stain densely with uranium salts are seen to correspond to segments of this dense annular structure surrounding the central hole. In general, the cross-sectioned TMV particles exhibit a circular or polygonal outline with an average diameter of 150 Å. However, in closely packed bundles there seems to be an intimate adherence of the outer layers of the particles, obliterating the individual outlines.

Ammonium vanadate and phosphotungstic acid

In longitudinal sections of oriented samples of TMV virus stained with aqueous solutions of ammonium vanadate or phosphotungstic acid the bundles of TMV particles packed in paracrystalline array appear as a regular system of parallel dense double bands uniformly separated by lighter spaces (Figs. 5, 6). Each of the dense double bands is 30–35 Å wide and shows indications of a granular fine structure of the order of 25–30 Å. In many areas the particulate components of adjacent double lines are found to be in lateral register, which may be due to orientation effects introduced by the preparation procedures. The light spaces between the bands are approximately 70–80 Å wide. When TMV specimens are stained first with uranyl acetate, and then with ammonium vanadate, a fine dense line of 20–25 Å is commonly encountered coursing through the middle of the light bands. It would appear therefore that there is a complementary relationship between the effects of the two types of reagents, the uranyl acetate staining the central region of the virus particles more intensely, while the ammonium vanadate emphasizes the outer layer.

Discussion

Interpretation of these preliminary findings is subject to the numerous sources of artefacts inherent in the preparation procedures. The described techniques furnish supplementary information, since the contrast introduced is not due to the surface deposition effects operative in the usual dried and stained virus suspensions. Moreover, the thin sectioning methods make it possible to observe the fine texture of the virus particles in any desired plane, while displaying the repetitive features of internal structure which are present in regular paracrystalline arrays.

The general picture of a cylindrical particle with a hollow axial core surrounded by a characteristic
Das Verhalten der alkalischen und sauren Phosphatase in Zellkulturen aus Affenmieren nach Infektion mit ECHO-Virus Serotyp 9 und Poliovirus hominis Typ 1*

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Diese Verschiebungen der Phosphatase-Aktivität in Abhängigkeit von der Zeit sind mit der Virusaktivität in Zusammenhang zu bringen.

Die Aktivität der alkalischen Phosphatase (AP) und der sauren Phosphatase (SP) verschiedener Organate verändert sich nach Infektion mit bestimmten Virusarten. So führt die Infektion mit Coxsackie-Viren der Gruppe A — Typ 1, 2 und 3 — zu einer Aktivitätssteigerung von AP und/oder SP im Muskel von Säuglings- oder ausgewachsenen Mäusen. Die Zeitspanne zwischen Infektion und Untersuchung ist dabei von ausschlaggebender Bedeutung. Im Rahmen solcher Beobachtungen konnte unerwarteterweise nachgewiesen werden, daß der maus-adaptierte Stamm Lansing des Poliovirus hominis Typ 2 bei intracerebraler Verimpfung auf Säuglingsmäuse am Nervengewebe bei nichtgelähmten Tieren einen deutlichen positiven Ausfall der AP hervorrief. Im Gegensatz hierzu verursachte die Infektion mit dem PR8-Influenzavirus eine signifikante Herabsetzung der AP-Aktivität im Dünndarm der Maus. Leider ist das Studium des Effektes von Virusinfektionen auf die Enzymaktivität und andere Stoffwechsel-

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3 W. ALBRECHT, Z. Kinderheilkunde 79, 270 [1957].