ELECTRON MICROSCOPY OF THEILER'S VIRUS, STRAIN FA

by

H. LEYON AND S. GARD, with technical assistance of G. EKLUND

The Institute of Physical Chemistry and Biochemistry, Uppsala, and the Department of Virus Research, the Caroline Institute, Stockholm (Sweden)

The morphology of poliomyelitis and related viruses has been a matter of some controversy. In 1941 GARD AND PEDERSEN[1], on the basis of physico-chemical measurements on purified specimens of THEILER's mouse encephalomyelitis virus (strain FA), assumed an asymmetrical shape of the virus particles, the dimensions being estimated at 640·14 mμ*. Later TISELIUS AND GARD[2], and GARD[3] observed in electron micrographs of purified material filaments with a width of 15–20 mμ. Similar structures were described by JUNGEBLUT AND BOURDILLON[4] in untreated tissue culture fluid of SK virus, whereas purified mouse brain virus showed approximately spherical particles of 25–30 mμ diameter. LORING, SCHWERDT, AND MARTON[5], applying a purification technique, in which treatment at low temperature for considerable periods of time entered as an essential feature, obtained the MV strain of poliomyelitis virus in the shape of spherical particles of 15–20 mμ diameter. LORING[6] observed the same morphology in the LANSING and YSK strains. Finally GOLLAN AND MARVIN[7] have published similar results with the MM virus/diameter 10–20 mμ/.

During work on the p_H-stability of the FA strain of THEILER's virus, the results of which will be reported later, some of our purified virus preparations were examined in the electron microscope. Since the micrographs obtained seem to us to be of some interest we wish to publish them here separately.

The virus was concentrated and purified by a combination of the methods previously used by BOURDILLON[8], GARD[3] and LORING AND SCHWERDT[9]. Frozen brains of mice infected with virus (40 g) were ground in a Waring blendor to obtain a 10% suspension of brain in 0.3% sodium chloride, the solution also containing 0.1% glycine. After centrifuging at low speed and treatment with ether the proteins were precipitated in 0.1 molar acetate buffer, p_H 4.5. The precipitate was resuspended in 0.1 molar phosphate buffer, p_H 7.5, to one tenth of the original volume. After centrifuging at a low speed the solution was brought to 33% saturation with ammonium sulphate at about + 4° C, the precipitate was centrifuged for 3 hours and was then resuspended in 0.001 molar phosphate buffer at p_H 7.5. The preparation was dialysed against distilled water over night and was spun in a Beams' centrifuge, 16000 rpm up and down and at 27000 rpm for 1½ hours. This centrifuging at low and high speed was repeated once. The pellets were left over night under a small volume of 0.001 molar phosphate buffer, p_H 7.5. After storing at —16° for about 2 months the preparation was thawed and was again spun at 16000 rpm up and down and at 27000 rpm for 90 minutes. After the last centrifuging no pellets could be seen. About 1 ml of the solution was left in the tube over night. If

* On the basis of repeated measurements the figures were subsequently adjusted to 580·12.5 mμ.

References p. 390.

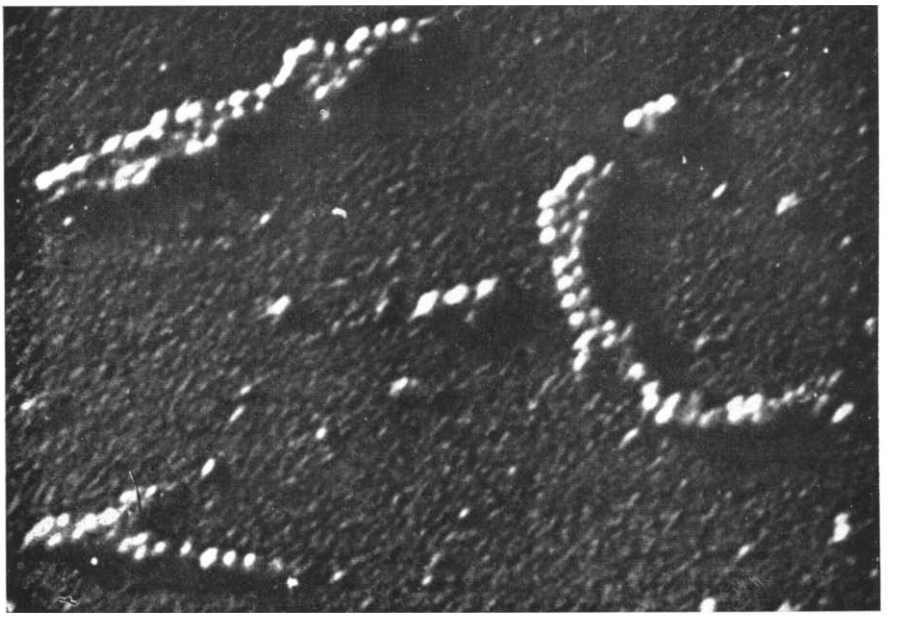

Fig. 1. A purified preparation of THEILER's virus, strain FA, goldshadowed (90000 ×)

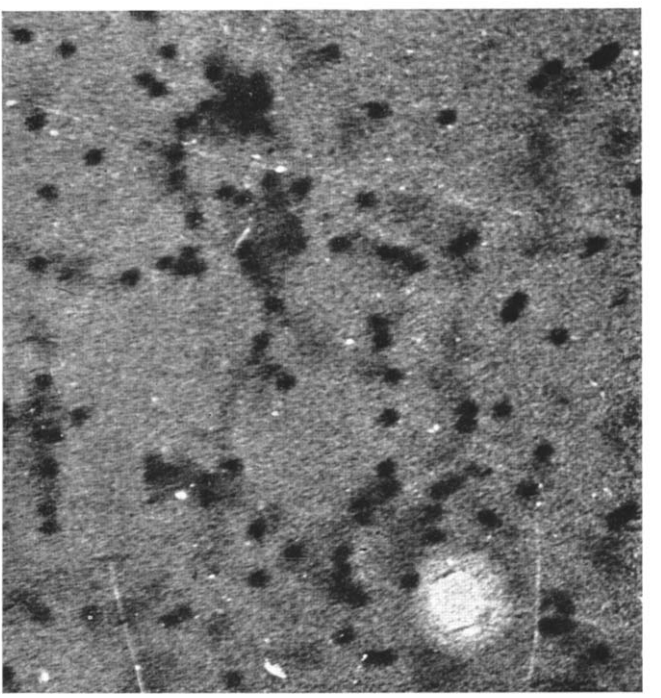

Fig. 2. A purified preparation of THEILER's virus, strain FA, treated with phosphotungstic acid (75000 ×)

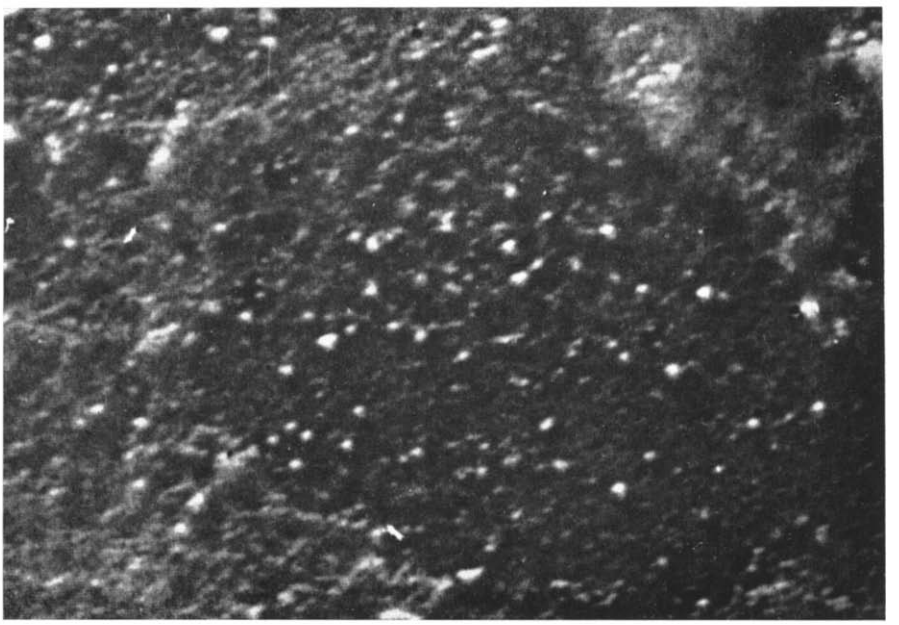

Fig. 3. A preparation from brains of mice which were not infected by the virus, gold-shadowed, magnification 90000 ×. The microgram shows particles ressembling those of the virus.

was necessary to dilute this preparation 100:1 to get some spots of the bottom free from material when working with the electron microscope.

The preparation was shadowed with gold and studied at a magnification of 8500. 10 electron micrographs were taken. They all showed but one structure which could be related to the virus, *viz.*, spherical particles with a diameter of about 25 mμ.

The size of the particles was also determined by taking electron micrographs of a preparation which had been purified by ficin treatment, and repeated freezing, thawing, low and high speed centrifuging. The preparation was not shadowed with gold but was treated in the dry state with a 0.1% solution of phosphotungstic acid for some minutes to obtain a better contrast and thereupon it was washed with water (Fig. 2).

In the first-mentioned preparation, which seems to have been of considerable purity, the particles were strongly aggregated (Fig. 1).

The concentration of the virus was determined by titrating in mice and was calculated by the method of REED AND MUENCH giving log ID_{50} per ml = 9.5. The nitrogen content was not determined.

As no titration with respect to the virus was performed separately on the original crude suspension the yield of the preparation cannot be given exactly. From earlier and later experiments the yield was estimated to be 10–50% of the virus.

As a control a preparation was made from non-infected mouse brains. It was treated in the same way that has been described with regard to the virus preparation but it was not precipitated with ammonium sulphate and was stored in the frozen state for 10 days only. When examined in the electron microscope this preparation showed several structures, among them many spherical particles of the same order of size as the virus but more varied (Fig. 3). The virus particles, on the contrary, seemed to be

References p. 390.

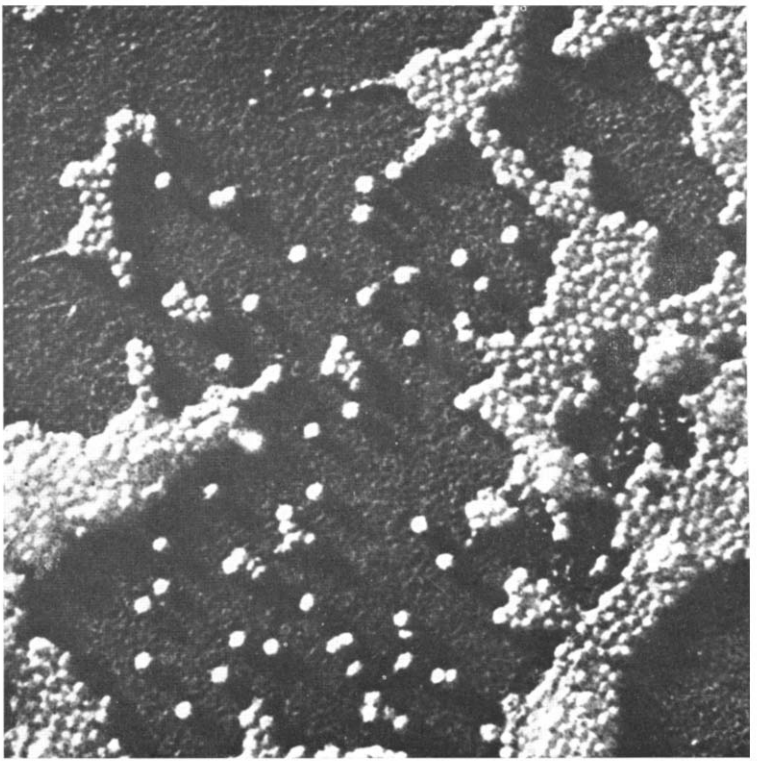

Fig. 4. A purified preparation of THEILER's virus, strain FA, gold-shadowed, magnification 60000 ×.

of the same size. After treatment with ammonium sulphate as well as after repeated freezing and thawing no particles resembling the virus could be seen in the electron microscope. The virus preparations, on the contrary, could be repeatedly frozen and thawed, seemingly without detriment.

On later occasions several virus preparations, purified by freezing and thawing have given results in accordance with those mentioned above when examined in the electron microscope (Fig. 4).

Thick metal shadow layers can cause considerable errors in the measurement of the size of these small particles. Individual systematic errors in the estimation of the blackening on the photographic plates can also give large discrepancies.

After different methods for shadowing and measurement had been conducted, it was found that the most reproducible results were obtained by the measurement of rows of aggregated particles in preparations shadowed by thin layers of metal. A measurement of 25 rows each containing 3–8 particles of a gold-shadowed preparation gave the size of the particle as 28.08 ± 0.01 mμ. The particle size was also determined by measuring the shadow length on the same plate, giving the size of the particle as 29.51 ± 0.75 mμ. On another occasion 10 measurements of rows of gold-shadowed particles from a similarly purified preparation gave a particle size of 27.08 ± 0.29 mμ. Of these measurements those of the shadow lengths are the most difficult and probably the least accurate.

References p. 390.

So, in this manner the particle size of spontaneous mouse encephalomyelitis virus, strain FA, purified by a procedure including repeated freezing and thawing can be estimated to be approximately 28 mμ.

DISCUSSION

Our results are in good agreement with those reported by JUNGEBLUT, LORING, AND GOLLAN, but deviate distinctly from the findings previously published by this laboratory. The reason for this latter discrepancy is not immediately clear. The virus preparations previously described by GARD appeared to be homogeneous on examination in the ultracentrifuge and showed an activity of at least the same order of magnitude as those reported by the other authors. The sedimentation constant was determined as 181 S. This figure conforms with the values found by one of us (G) in repeated experiments on LANSING, MM, FA, and TO viruses (unpublished results, presented at the IVth International Congress for Microbiology). Thus in brain extracts, clarified by centrifugation at 16000 rpm but otherwise untreated, the viral activity was found to sediment at a uniform rate, corresponding to $s_{20} = 195$ S, when analysed in the separation cell. Practically the same figure, approximately 200 S, was reported by BOURDILLON AND MOORE[10], using a sampling technique. On the other hand, LORING's preparation of the LANSING strain had a sedimentation constant of 83 S[9]. Our present preparation has not yet been analysed in the ultracentrifuge, so we cannot estimate its rate of sedimentation. LORING's figure, however, conforms well with the size of the particles as estimated from the electron micrographs.

At present we cannot offer any explanation of the discrepancies mentioned. It might be suggested, however, that the virus particles in the crude brain extracts appear in aggregates that are broken down into isolated elementary bodies on freezing or by treatment at a low p_H.

ACKNOWLEDGMENT

This work is part of an investigation of THEILER's virus, financially supported by a grant from the SWEDISH NATURAL SCIENCE RESEARCH COUNCIL.

The authors would like to thank Professor ARNE TISELIUS for his encouragement of the work and Professor THE SVEDBERG for the privilege of working in his Institute.

SUMMARY

Electron micrographs of purified THEILER's virus, strain FA, showed as only structural elements spherical particles with a diameter of 28 mμ.

RÉSUMÉ

Des photographies au microscope électronique de virus THEILER purifié, souche FA, montrent que les seuls éléments de structure sont des particules sphériques de diamètre 28 mμ.

ZUSAMMENFASSUNG

Elektronenmikrographien von gereinigtem THEILERSCHEN Virus, Stamm FA, zeigen als einziges Strukturelement kugelförmige Teilchen mit einem Durchmesser von 28 mμ.

References p. 390.

REFERENCES

1 S. GARD AND K. O. PEDERSEN, *Science*, 94 (1941) 493.
2 A. TISELIUS AND S. GARD, *Naturwissenschaften*, 30 (1942) 728.
3 S. GARD, *Acta Med. Scand., Suppl.*, 143 (1943).
4 C. W. JUNGEBLUT AND J. BOURDILLON, *J. Am. Med. Ass.*, 123 (1943) 399.
5 H. S. LORING, C. E. SCHWERDT, AND L. MARTON, *Phys. Rev.*, 65 (1944) 354.
6 H. S. LORING, *Proc. Soc. Exptl Biol. Med.*, 67 (1948) 366.
7 F. GOLLAN AND J. F. MARVIN, *Proc. Soc. Exptl Biol. Med.*, 62 (1946) 289.
8 J. BOURDILLON, *Arch. Biochem.*, 3 (1944) 285.
9 H. S. LORING AND C. E. SCHWERDT, *Proc. Soc. Exptl Biol. Med.*, 62 (1946) 289.
10 J. BOURDILLON AND D. H. MOORE, *Science*, 96 (1942) 541.

Received March 7th, 1949