transparent ultracentrifuge[5] have shown them to be relatively monodisperse as shown in Fig. 1 and to have a sedimentation rate of 83.5 ± 7.35 (average of 20 sedimentation runs on 5 preparations).

Conclusions. The results outlined above including (a) the presence in the purified samples of a uniform and high specific activity, (b) the demonstration that normal macromolecular constituents are largely eliminated by the purification procedures, and (c) the fact that the samples are relatively monodisperse in the transparent ultracentrifuge provide strong evidence that the preparations consist of essentially pure Lansing virus.

Presented at the Symposium of the Division of Biological Chemistry of the American Chemical Society on Biochemical and Biophysical Studies on Viruses at Atlantic City on April 12, 1946.

5 McBain, J. W., and Lewis, A. H., *J. Physical Chem.*, 1939, **43**, 1197.

15453 P

Electron Microscopy of Purified Lansing Virus.*

HUBERT S. LORING, L. MARTON, AND C. E. SCHWERDT.
(With technical assistance of Marjorie B. Glathe and Patricia Ruth Schwerdt.)
From the Department of Chemistry and School of Medicine and the Division of Electron Optics, Stanford University, Calif.

Eleven different samples of purified Lansing virus prepared as described in the preceding paper have been examined in the Stanford election microscope.[1] The preparations were obtained from extracts that had been frozen from 4 to 26 days and gave specific activities, $I.D._{50}$, from $10^{-10.1}$ to $10^{-8.3}$ g of virus nitrogen. Mounts were prepared by the usual technic of applying a small drop of dilute solution (10^{-8} to 10^{-7} g of virus nitrogen per ml) to the collodion film and allowing the solution to dry or by washing away the remainder of the drop with distilled water after 2 to 5 minutes. The mount was then either introduced directly in the microscope or it was treated by the gold shadowing technic of Williams and Wyckoff[2] before placing in the microscope.

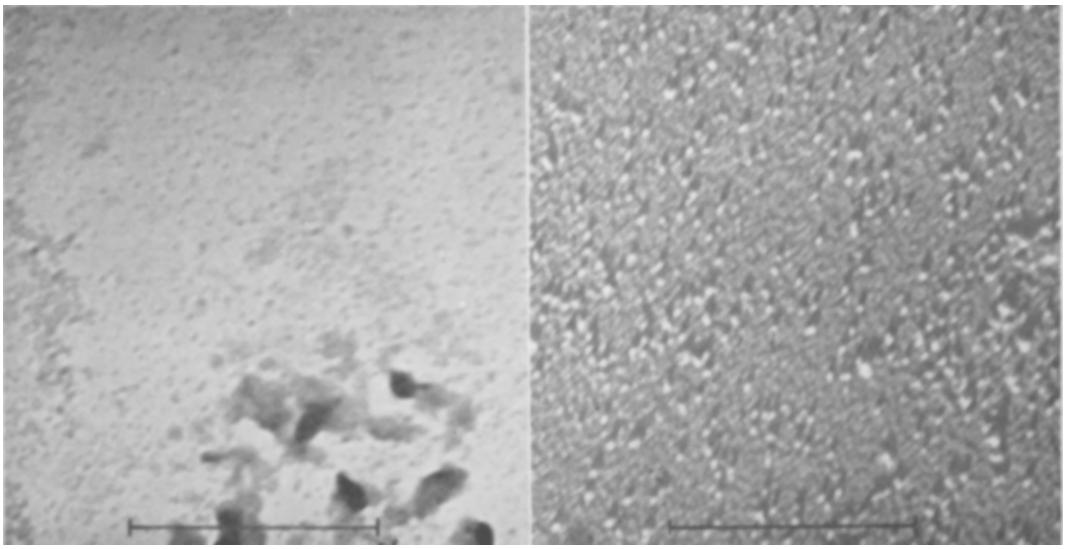
Although all the micrographs have not yielded uniform or conclusive results, they have in general shown the presence of spherical or possibly slightly asymmetrical particles ranging in size from 12 to 34 mμ. Examples of micrographs obtained when the virus was examined without gold shadowing as well as of those obtained by the gold-shadowing technic are shown in Fig. 1a and 1b. Fig. 1a was obtained from an extract that had been frozen for 26 days and purified by 2 cycles of ultracentrifugal purification and Fig. 1b from an extract frozen for 4 days and purified by 3 cycles of ultracentrifugal purification, the specific activities being $I.D._{50} = 10^{-9.1}$ in both cases. Fig. 1a was obtained by the usual technic and Fig. 1b after gold shadowing.

In Fig. 1a there is some indication of slightly asymmetric particles with an average width of about 16 mμ and an average length of about 31 mμ or an average particle diameter of 23 mμ. In Fig. 1b this is not so apparent and the range of particle size as measured by shadow width is from about 15 to 34 mμ with an average value for particle diameter of 25 mμ. The slightly lower value for the non-shadowed specimen can probably be explained by the relatively low contrast between particles and background with the accompanying lack of definition in the particle image. Under these conditions the measured dimension is the greatest distance within the particle image of points of appreciable pho-

* Aided by grants from the National Foundation for Infantile Paralysis and the Rockefeller Foundation.

1 Marton, L., *J. Applied Physics*, 1945, **16**, 131.

2 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.



1a 1b

Fig. 1.

Electron micrographs of purified Lansing virus. The inscribed line in each case has a length corresponding to one micron. 1a. Concentration of 10^{-7} g virus nitrogen per ml applied to film. 1b. Same concentration as in 1a, mount placed in microscope after gold shadowing.

tographic contrast, which may not coincide with the true edges of the particle. In the case of the gold-shadowed specimens the shadow width is the measured dimension and because of the high degree of contrast this can be determined with considerably greater accuracy.

Of particular interest is the absence in all of the purified Lansing virus preparation of asymmetrical or thread-like particles as found by Gard[3] for both murine (Theiler's virus) and human poliomyelitis virus and as suggested by Bourdillon[4] for the SK mouse virus. While the results of Gard appear conclusive in the case of Theiler's virus, his conclusions with respect to human poliomyelitis were admittedly based on only a few experiments and open to other interpretations. The latter purified preparations from both infected tissues and feces contained components with sedimentation rates of the order of magnitude described in the preceding paper for the Lansing virus. Similarly while some filamentous particles were observed in his electron micrographs, there was also ample evidence for small, approximately spherical, particles. We feel, therefore, that Gard's results on human poliomyelitis are not necessarily incompatible with those presented here.

Conclusions. The results mentioned in the preceding paper as well as those given here lead to the conclusion that the Lansing virus, unlike Theiler's virus and probably the SK mouse virus, is a relatively spherical or slightly asymmetrical particle of about 25 mμ average particle diameter. It may be recalled that a somewhat similar conclusion was reported for the MV virus.[5] It appears to the present authors that such values for the size of these strains of poliomyelitis virus of human origin are also more in accord with the filtration end-point data and the conditions that have been found necessary for sedimentation in the ultracentrifuge.

Presented at the Symposium of the Division of Biological Chemistry of the American Chemical Society on Biochemical and Biophysical Studies on Viruses at Atlantic City on April 12, 1946.

[3] Gard, S., *Acta Med. Scand., Suppl.*, 1943, **143**, 173p.

[4] Bourdillon, J., *Arch. Biochem.*, 1943, **3**, 285.

[5] Loring, H. S., Schwerdt, C. E., and Marton, L., *Physical Rev.*, 1944, **65**, 354.