Table I. In this experiment a 43-fold purification was achieved and after the deduction of the 35% loss of virus activity a purification factor of about 28 resulted.

Conclusion. A simple method requiring standard laboratory equipment only is described which by means of freezing and thawing, alcohol precipitation and dialysis accomplishes a marked purification of the MM poliomyelitis virus.

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Electronmicroscopy of the Purified MM Poliomyelitis Virus.*

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The purified virus used for electronmicroscopic studies was prepared in essentially the same way as described in detail in the preceding paper. During the preliminary studies the observation was made that highly concentrated brain suspensions in phosphate buffer yielded heavier precipitates after freezing and that a higher degree of purification could be achieved by keeping the protein concentration as high as possible.

A 25% mixture of infected mouse brain and cord in 0.1 M phosphate buffer pH 7.0 was prepared by homogenizing the previously frozen tissue in a Potter-Elvehjem glass homogenizer. The mixture was then frozen for 24 hours. After thawing the tissue particles were centrifuged off, the clear supernate was extracted with two-thirds its volume of ether, centrifuged and the gelatinous layer at the surface was discarded. The remaining ether was removed in a desiccator and the solution was frozen overnight. After thawing the precipitate was centrifuged off and discarded. The temperature was then lowered to $-1°C$ and enough chilled methyl alcohol was added to a final concentration of 30% methanol. After 6 hours the precipitate was centrifuged off, washed once with 30% methyl alcohol in buffer, suspended in one-tenth of the original volume, the insoluble particles were centrifuged off and the supernate was frozen overnight. After thawing the precipitate was centrifuged off and discarded. The supernatant fluid was dialysed against distilled water for 24 hours, the water insoluble proteins were centrifuged off and discarded. The supernatant fluid was frozen for 2 weeks, thawed and a final precipitate was centrifuged off and discarded.

By this procedure the nitrogen content was reduced from 2.72 mg per ml in the original clear solution to 0.0119 mg per ml in the final fraction. Thus, a 228 fold purification was achieved or 99.5% of the total nitrogen of the original solution was removed. The final yield amounted to about 10% of the total virus activity of the original solution and therefore a purification factor of about 22.8 resulted. The final fraction contained 1.19×10^{-12} g of "virus" nitrogen per ml and one inoculum contained 3.9×10^{-14} g of "virus" nitrogen.

The mounts for the electron micrographs were prepared by the usual technique of applying a small drop of the 10 times diluted final fraction (1.19×10^{-13} g nitrogen per ml) to a thin formvar membrane supported by a 1/8 inch diameter disc of 200 mesh copper screen and allowing it to dry. The gold-shadowed specimen was prepared by applying a small drop of the solution to a microscope slide. This was treated by the gold shadowing technique of Williams and Wyckoff[1] (8 AU film of gold at a shadow angle of 5 to 1) stripped with collodion and mounted on the copper screen. Measurement of the par-

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

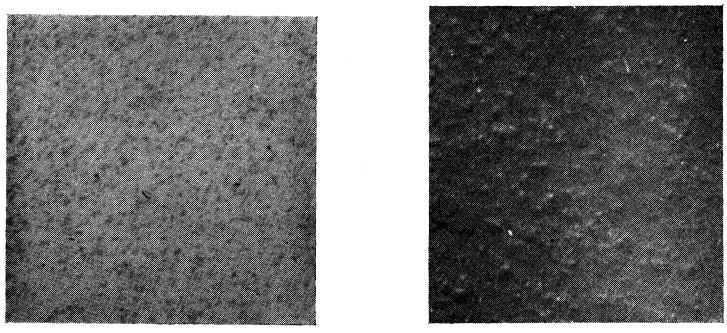

FIG. 1.
Electron micrographs and shadowgraphs of an infected mousebrain suspension containing 3.9×10^{-14} g nitrogen per LD_{50}. Magnification 30,000.

ticle dimensions could be made by measuring the shadow width and shadow length. The shadow length should be 5 times the diameter.

In both micrographs there is an indication for a slight asymmetry of the particles. In measuring the diameter of the particles a minimum dimension of 10 mμ and a maximum of 20 mμ with an average of about 12 mμ was found.†

These micrographs confirm the findings of Loring, Marton and Schwerdt[2] of the absence of thread-like particles[3] and show also essentially the same shape and size of particles as described by these authors.

Summary. Electronmicroscopic studies of a preparation of purified MM Poliomyelitis virus show an uniformity of particle size in the range from 10 to 20 mμ with an average diameter of about 12 mμ.

† These measurements would indicate a molecular weight of the order of 500,000 to 1,000,000.

2 Loring, H. S., Marton, L., and Schwerdt, C. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 291.

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

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Serum and Plasma Antithrombin.*

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For years it has been known that blood has the ability to inactivate added, as well as spontaneously formed, thrombin. Rettger[1] attributed this to the plasma proteins as a specific function; Lenggenhager[2] related the activity to the albumin fraction. Smith's[3] group has suggested that the *rate* of thrombin destruction varies with the heparin concentration and that the absolute *amount* is limited by the concentration of heparin "cofactor." Wilson[4] developed a method for estimating antithrombin by so diluting plasma

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1 Rettger, L. J., *Am. J. Physiol.*, 1909, **24**, 406.

2 Lenggenhager, Karl, *Helv. med. Acta*, 1935, **1**, 527.

3 Seegers, W. H., Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Science*, 1942, **96**, 300.

4 Wilson, S. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 676.