kept at 4°C, and infectivity measurements were made at intervals of 1, 8, 15 and 29 days.

Virus growth was studied in embryos of 7, 8 and 9 days incubation at 37.5°C before inoculation; hemagglutination was measured daily after the 4th day of incubation at 35°C until a total incubation of 15 days. Infectivity measurements were made in parallel with the fluid from the eggs 7 days old at inoculation. The influence of concentration of virus in the inoculum was examined with dilutions of chorio-allantoic fluid of 10^{-0}, 10^{-1}, 10^{-2} and 10^{-3}.

Results. There was no evidence of inactivation *in vitro* of the virus by the antibiotics. Penicillin[3] and streptomycin in concentrations of 500 units and 100 μg per cc introduced with the inoculums (0.05 cc) did not hinder virus growth in the egg.

The greatest increase in virus hemagglutinative capacity occurred in 7-day eggs with the hemagglutinative units per cc and total hemagglutinative units increasing to the 8th day after inoculation. Infectivity in 7-day embryos reached a maximum at the 4th to 6th days after inoculation and diminished thereafter. Infectious titers per cc of pooled fluids have varied from $10^{-8.5}$ to $10^{-9.5}$ and the hemagglutination units (2 +) from 45 to 128 per cc. Titers of individual eggs vary widely, from 22 to 360. The concentration of virus in the inoculum was not an influencing factor.

The mumps virus has been very unstable under any conditions thus far studied. Titers in chorio-allantoic fluid at 4°C diminished as much as from $10^{9.0}$ to $10^{7.4}$ infectious units per cc within 2 weeks. The range of optimum pH stability was from about pH 5.65 to 7.9. Maximum stability appeared to be at pH 6.5 to 7.0. Inactivation was very rapid below pH 4 and above pH 9.5. Little inactivation occurred within 8 days in the range of pH 5.65 to 7.9; after 29 days the titers in this range were about 2 10-fold dilutions lower than the initial level.

Summary. Neither penicillin nor streptomycin, separately or combined, caused demonstrable inhibition of the mumps virus *in vitro* or *in vivo*. Maximum virus infectivity occurred in 7-day-old embryos 4-6 days after inoculation; hemagglutinative capacity reached a maximum in like embryos after 8 days of incubation. The concentration of virus containing chorio-allantoic fluid used as inoculum had no apparent effect on growth of the virus in eggs. The range of optimum pH stability was 5.65 to 7.9, but the virus was relatively unstable under any conditions.

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Purification and Sedimentation and Electron Micrographic Characters of the Mumps Virus.*

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In an accompanying paper,[1] there are described the results of experiments on the growth and stability of mumps virus carried out preliminary to studies on the purification of the virus. The purification and the sedimentation and electron micrographic characters of the agent are described here.

Material and Methods. The general methods employed in the work have already been described.[1] The crude chorio-allantoic fluid harvested 5 days after inoculation of 7-day-old chick embryos, and held at 4°C for 1-4 weeks, was spun in an angle centrifuge at 1,700

* This work was aided by a grant to Duke University from Lederle Laboratories, Inc., Pearl River, N.Y.

1 Weil, M. L., Beard, D., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 308.

g for 15 minutes. The supernate was then filtered through No. 1 Whatman filter paper and spun in the horizontal head at 3,000 g for 1 hour. This supernate was centrifuged at 10,000 g for 1 hour to sediment the virus, and the pellets were resuspended in ½ the initial volume in half strength Ringer solution containing glycine in 0.01 M concentration. This suspension was then spun at 10,000 g for 5 minutes, and the resulting supernate centrifuged again at 10,000 g for 45 minutes. The second cycle pellets were resuspended at 56 × concentration in Ringer-glycine solution. The suspension was then centrifuged in the angle head at 625 g for 10 minutes. The supernate contained the concentrated and purified virus. All manipulations were carried out in the cold. Sedimentation studies were made with the air-driven analytical ultracentrifuge using the absorption method of Svedberg and also the Lamm scale procedure.[2] The electron microscope was the RCA type B instrument.[3]

Results. The average yield of chorio-allantoic fluid has been 5-6 cc per egg and the pH of pooled fluids about 8. Filtration through filter paper seems to remove mucoid and other extraneous material. The sedimented virus is very difficult to redisperse. Aggregation occurs readily in 0.9% NaCl; 0.005 to 0.05 M phosphate buffer; and in Ringer solution. The best results were obtained with the dilute Ringer-glycine solution of pH 7.4. The pellets of the first sedimentation contained a central area of yellowish material which redispersed with the virus. Some of this was eliminated in the brief centrifugation at 10,000 g. The pellets of the second sedimentation were nearly clear, containing little opaque material.

The final yield of hemagglutinative factor was from 31-60% of the starting material; the virus infectivity recovered was 5-13%. The chief losses in infectivity appeared to be related to inactivation of the virus in the centrifugal procedures. The 2+ hemagglutinative unit was $10^{-7.19}$ g of nitrogen and the infectious unit for chick embryo was $10^{-13.76}$ g of nitrogen. The yields were from 0.25 to 0.45 mg of virus nitrogen per 100 cc of chorio-allantoic fluid.

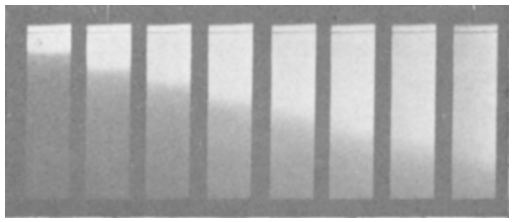

FIG. 1.
Sedimentation diagram of mumps virus. The concentration was 143 γ of virus nitrogen per cc. The interval between exposures was 3 minutes and the centrifugal field 4,100 g.

Fig. 1 is the sedimentation diagram of a preparation containing 143 γ of virus nitrogen per cc. The broad spread in the boundary indicates a wide variation in particle size which is confirmed by the electron micrographs. A similar spread in boundary was seen in the Lamm scale diagrams. Measurements of successive peaks gave an average sedimentation rate of $S_{20}^{\circ} = 1330 \times 10^{-13}$ for the virus.

Much difficulty has been experienced in obtaining electron micrographs. The images of the agent in the electron microscope have been indefinite and are of irregular shape. Micrographs of purified virus treated for 31 days with 0.1% formalin are shown in Fig. 2 and 3. The images under these conditions appear approximately circular and exceedingly variable in size ranging from 106 to 282 mμ with an average of 190 mμ and a standard deviation of 42 mμ. There is only a suggestion of internal material of high absorbing power for electrons. In the shadowed[4] micrograph there is a great similarity between individual particles although the same variation in image size is seen. The particles are flattened and show a roughened, irregular surface contour somewhat similar to the elementary bodies of vaccinia.[5] The shape of the formolized virus

[2] Svedberg, T., and Pedersen, K. O., *The Ultracentrifuge*, Oxford, 1940.

[3] Hillier, J., and Vance, A. W., *Proc. Inst. Radio Engrs.*, 1941, **29**, 267.

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

[5] Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 295.

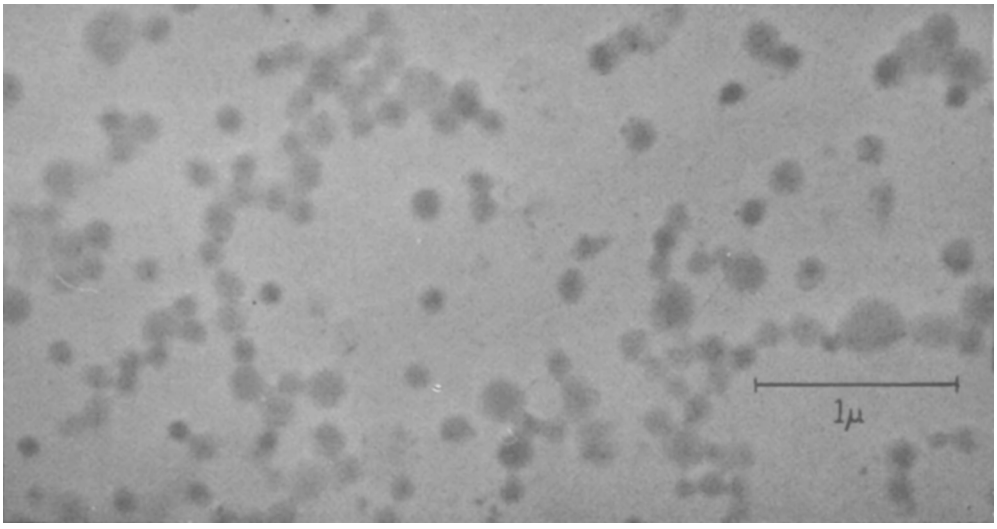

FIG. 2.
Electron micrograph of a mumps virus concentrate after treatment with 0.1% formalin for 31 days. Magnification = 27,320 ×.

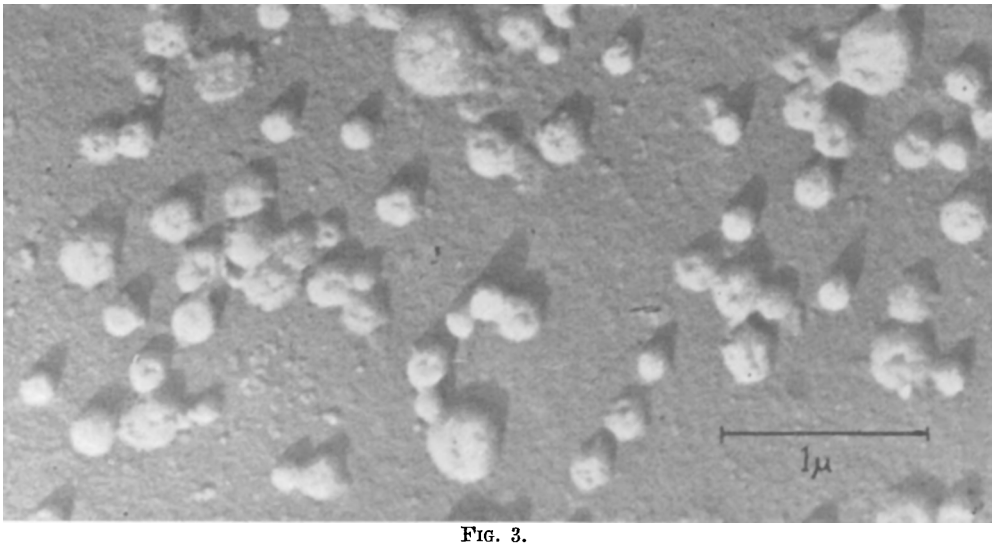

FIG. 3.
The virus preparation of Fig. 2 shadowed with 19 A° of chromium at an angle whose tangent was 2/8.5. Magnification = 28,800 ×.

was inferred to be spherical. This preparation showed relatively very few of the small particles the size of the normal component of chorio-allantoic fluid.

Summary. The mumps virus has been purified by centrifugation of virus-infected chorio-allantoic fluid. Sedimentation diagrams indicate wide variation in particle size and a sedimentation rate of about 1330 × 10^{-13}. The electron micrographs corroborate the centrifugal evidence of variation in particle size. The shape of the formolized virus was judged to be spherical and flattened electron micrograph images have a diameter of 190 ± 42 mμ.