

STUDIES ON THE NATURE OF THE VIRUS OF INFLUENZA

II. THE SIZE OF THE INFECTIOUS UNIT IN INFLUENZA A

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PLATE 14

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The size of the pathogenic agent of influenza A has, until recently, been generally accepted as that of a sphere about 100 $m\mu$ in diameter. The virus-containing preparations used in both the ultrafiltration (1) and the centrifugation experiments (2) upon which this estimate was based, consisted of emulsified lung tissue from infected mice.

In the preceding paper two of us have shown that particles having an average diameter of 100 $m\mu$ can be obtained in essentially equal quantities from both normal and infected lungs (3). The infectious and non-infectious particles showed no significant difference in chemical composition, density, spectral absorption, staining properties, or electron microscopic appearance. This suggested that some particulate element of normal tissues acts as carrier for a considerably smaller infectious agent. Strong support for this theory was obtained when the extra-embryonic fluids of chicks infected with the virus of influenza A were found to contain the virus in high concentration although both normal and infective fluids were essentially free from particles approaching 100 $m\mu$ in diameter (4). It was found that the virus existed in such a state in the egg fluids that it frequently could not be sedimented at centrifuge speeds which removed the activity almost entirely from mouse lung filtrates. It was also shown that 100 $m\mu$ particles separated from normal lungs can absorb the virus almost completely from the egg fluid and thereby acquire the infective property. Thus clear evidence was obtained that normal lung constituents *can* act as passive carriers of a smaller virus unit.

From semiquantitative data obtained with the concentration centrifuge, and from considerations based on the infectivity titers in relation to the total egg fluid protein, it was suggested that the elementary virus particle is probably not much more than 10 $m\mu$ in diameter (*cf.* 5).

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This conclusion is in fair agreement with the results of Bourdillon (6) who reported on the diffusion velocity of the influenza virus, which he interprets as indicating a unit diameter of about 6 μ . His method has been strongly criticized, but the estimate of particle size obtained by him appears to be within a factor of 2 on a diameter basis and a factor of 10 on a weight basis of the order of magnitude indicated above.

The present paper contains the results of experiments designed to obtain more definite evidence concerning the size of the influenza virus as it occurs in the pooled allantoic and amniotic fluids of infected chick embryos.

Materials and Methods

Source of Virus.—10-day-old chick embryos were inoculated with 0.5 ml. of a suitable dilution of the F-12 strain (7) of influenza A virus from the 32nd egg passage in a manner previously described (4). After 24 hours' incubation at 39°C., the eggs were refrigerated for 1 to 2 hours and the extra-embryonic fluids harvested with care to prevent undue contamination with blood (*cf.* 8*a* and *b*). Most of the material used was collected from embryos which were living when placed in the refrigerator. The fluid was clarified in the horizontal centrifuge and kept at -10 to -15°C., overnight. Immediately prior to the ultracentrifugation the fluids were again clarified in the Swedish angle centrifuge. This possibly removed a sizable portion of the virus (*cf.* 9).

Centrifugation.—Two concentration ultracentrifuges of the air turbine type were used for initial concentration and washing of the virus. The dimensions of the rotors and their capacities differed, but an average centrifugal field of about 90,000 *g* was obtainable in each. After centrifugation of the clarified fluids, the upper 8 ml. was carefully removed from each tube and pooled for protein and activity determinations. The remaining 5 ml. was poured off and discarded. Sedimented pellets were resuspended in 0.85 per cent NaCl solution buffered with 0.01 *M* phosphate at pH 7.0, and run in a Swedish angle centrifuge for about 20 minutes to remove any large particles. The analytical centrifuge used, as well as the concentration centrifuges, were those of the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Estimation of Protein.—Protein nitrogen in the various fluids and resuspended sediments was estimated by Kjeldahl analyses of the precipitates resulting from the addition of an equal volume of hot 10 per cent trichloroacetic acid. A nitrogen-protein conversion factor of 6.25 was employed.

Virus Titrations.—Serial tenfold dilutions of specimens from various stages in the concentration procedure were made in broth and each of a group of 4 Swiss mice was inoculated intranasally under light ether anesthesia with 0.05 ml. of one of the dilutions. Those animals which died within 10 days were autopsied and their lungs examined for the presence of typical consolidation. At the end of the experimental period the surviving mice were sacrificed and the extent of the lung lesions noted as 4 = complete consolidation; 3 = $\frac{3}{4}$; 2 = $\frac{1}{2}$; 1 = $\frac{1}{4}$ consolidated; \pm = very small lesion; and 0 = no lesion.

Electronmicrographs.—Specimens for electronmicroscopy were prepared by drying small drops of suitable aqueous dilutions of the virus on thin collodion films or by

adsorbing the virus from dilute solution onto such films, the excess solution being flooded away with distilled water before drying of the mount. An RCA type B electron microscope was used in the Laboratories of the RCA Manufacturing Company, Camden, New Jersey, during early phases of the study. Recently an instrument of the same type has been used in the laboratories of the Johnson Research Foundation.

EXPERIMENTAL

Sedimentation of the Virus in the Ultracentrifuge As an Indication of Virus Size

Five different lots of clarified allantoic fluid from chick embryos inoculated 18 to 48 hours previously with the F-12 strain of influenza A served as starting material for the experiments to be reported. In each instance the fluids were stored overnight at -10 to -15°C ., transported from Philadelphia to Princeton the following morning, and reclarified in the Swedish angle centrifuge before distribution in the Lusteroid tubes of the ultracentrifuge rotor. Sufficient samples of the starting material and of the successive fractions obtained during each run were taken for chemical analysis and for infectivity titration. In each case the ultracentrifuge sediments were taken up in the smallest practicable amount of 0.85 per cent NaCl solution buffered at pH 7.0 and large aggregates not readily dissolvable were removed by brief low speed centrifugation. After collection, the various samples were returned to Philadelphia for titration and analysis on the same or following day.

In the first experiment 156 ml. of allantoic fluid was first run for 90 minutes in a centrifugal force field approximating 50,000 times gravity (run IA). The supernatant liquid from IA was then subjected to 90,000 times gravity for an equal period of time. Included in run IA were one tube each of two different concentrations of purified tobacco mosaic virus (TMV), 5.2 and 0.7 mg. per ml. respectively, suspended in 0.1 M phosphate buffer at pH 7.0. Tests conducted by Dr. W. M. Stanley showed that the tobacco mosaic virus was almost completely thrown out of suspension by the relatively low force. Run IB included one tube containing 1.15 mg. per ml. of crystalline ovalbumin suspended in water. Only about 0.6 per cent of the protein was sedimented. Since, as indicated in Table I, only a portion of the infectivity of allantoic fluids was removed at the low, and almost all of it at the high speed, it was evident that the sedimentation constant of the virus fell somewhere between that of tobacco mosaic virus and ovalbumin. In other words, the sedimentation velocity sought was that of a spherical particle having a diameter less than $60\text{ m}\mu$, but greater than $4.5\text{ m}\mu$. This preliminary information confirmed earlier findings and provided an estimate of speeds to be employed in subsequent determinations of sedimentation velocity in the analytical ultracentrifuge.

Table I shows the results of infectivity titrations of the original fluid and the successive sediments and supernatant fluids from experiment I. It is evident that less than half of the virus was sedimented at 50,000 times gravity. After removal of this sediment, the upper two-thirds of the supernatant fluid from each tube was subjected to 90,000 times gravity for 90 minutes whereupon all but a very small fraction of the virus was found in the resuspended sediment.

Table II contains data on the amount of protein sedimented from the allantoic fluids in each of the two centrifuge runs. A total of about 0.07 mg. per ml. was removed altogether of which 0.03 mg. was thrown down in run IA and the remaining 0.04 mg. in run IB. This distribution approximates the distribution of virus in the two sediments as indicated in Table I. No measurable amount of protein was removed from normal, uninfected allantoic fluid from chicks of the same age as those from which the virus was harvested, by centrifugation at 90,000 times gravity. Thus it appears probable that all the sedimentable material is either virus or is present as a consequence of the infection.

In the final column of Table II an attempt has been made to estimate the weight of an infectious dose of the two sediments from the results of the pro-

TABLE I
Sedimentation of the Infectious Agent from Extra-Embryonic Fluid

| Dilution | Original fluid | IA 50,000 g | | IB 90,000 g | |
|-------------------|---|---|---|---|---|
| | | Supernatant | Sediment concentration 6:1 | Supernatant | Sediment concentration 25:1 |
| 10 ⁻² | D ₄ D ₄ D ₆ D ₇ | D ₄ D ₄ D ₄ D ₅ | D ₄ D ₅ D ₅ D ₇ | D ₅ D ₆ D ₇ D ₈ | D ₄ D ₄ D ₄ D ₄ |
| 10 ⁻³ | D ₄ D ₅ D ₅ D ₈ | D ₄ D ₅ D ₅ D ₅ | D ₄ D ₄ D ₅ D ₆ | D ₇ D ₉ D ₉ 4 | D ₄ D ₄ D ₄ D ₅ |
| 10 ⁻⁴ | D ₅ D ₅ D ₅ D ₈ | D ₆ D ₈ D ₈ D ₈ | D ₅ D ₅ D ₆ D ₉ | 3 2 2 2 | D ₄ D ₆ D ₆ D ₉ |
| 10 ⁻⁵ | D ₆ D ₆ D ₇ D ₇ | D ₆ D ₇ D ₈ D ₈ | D ₅ D ₆ D ₇ 3 | 2 2 2 0 | D ₅ D ₆ D ₆ D ₇ |
| 10 ⁻⁶ | D ₈ D ₉ D ₁₀ 3 | D ₉ D ₁₀ 3 3 | 3 3 3 2 | 1 1 0 0 | D ₇ D ₈ 3 0 |
| 10 ⁻⁷ | 4 3 3 0 | 3 2 1 ± | 3 1 1 ± | 0 0 0 0 | 4 3 3 2 |
| 10 ⁻⁸ | 2 2 ± 0 | D ₁₀ 1 ± 0 | 1 0 0 0 | 0 0 0 0 | 2 1 0 0 |
| 10 ⁻⁹ | 1 1 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 1 0 0 0 |
| 10 ⁻¹⁰ | 1 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 1 0 0 0 |

tein determinations and the mouse titrations. The figures indicate that about 10⁻¹³ gm. of either preparation was capable of producing lung lesions in mice with sediment IB.

The results of the other four centrifuge experiments (II-V) are also summarized in Table II. In these experiments the major objective was concentration of the virus for study in the analytical centrifuge. Since the preliminary evidence indicated that the low and high speed sediments showed about the same weight/infectivity ratio and, therefore, consisted of the same sort of material, the low speed step was not employed and the fluids were centrifuged only at 90,000 times gravity.

Table III contains the results of the infectivity titrations carried out on the various samples taken during one of these experiments (II), and is fairly representative. In each instance the removal of the virus from the allantoic fluid was so complete that only a very small fraction of 1 per cent remained in the supernatant fluid.

Table II shows the original quantity of fluid used in each experiment, the amount of protein recovered in the washed ultracentrifuge sediments, the amount contained in the resuspension used in mouse titration, and an approximation of the weight of sediment protein which produced lung lesions in mice.

TABLE II
Yield of Virus and Weight of Minimal Infectious Dose

| Run | RCF (× gravity) | Age of culture | Original fluid | Total protein recovered after washing | Final protein concentration | MID50* | Minimal infectious dose |
|-----|--------------------|-------------------|-------------------|---|-----------------------------------|---------------------|----------------------------|
| | | <i>hrs.</i> | <i>cc.</i> | <i>mg.</i> | <i>mg./cc.</i> | | <i>gm.</i> |
| IA | 50,000 | 24 | 156 | 4.175 | 0.167 | 10 ^{-7.7} | 1.67 × 10 ⁻¹³ |
| IB | 90,000 | 24 | 78 | 1.56 | 0.52 | 10 ^{-8.2} | 1.64 × 10 ⁻¹³ |
| II | 90,000 | 24 | 360 | 6.30 | 2.10 | 10 ^{-11.5} | 3.3 × 10 ⁻¹⁶ |
| III | 90,000 | 18 | 550 | 1.62 | 0.54 | 10 ^{-10.5} | 8.6 × 10 ⁻¹⁶ |
| IV | 90,000 | 24 | 720 | 5.08 | 0.705 | 10 ^{-9.4} | 1.4 × 10 ⁻¹⁴ |
| V | 90,000 | 48 | 360 | 9.09 | 6.06 | 10 ^{-9.9} | 5.1 × 10 ⁻¹⁴ |

* MID50 = maximal dilution producing lesions in 50 per cent of the test animals.

TABLE III
Sedimentation of the Infectious Agent (Experiment II)

| Test | Dilution of sample | Original | Supernatant fluid | Sediment concentration 125:1 | Sediment plus anti-PR-8 serum |
|-----------------|-----------------------|--|---|---|--|
| Mouse titration | 10 ⁻³ | D ₈ D ₃ D ₆ D ₆ | D ₆ D ₆ D ₇ D ₇ | D ₄ D ₄ D ₅ D ₇ | 1 0 0 0 |
| | 10 ⁻⁴ | D ₃ D ₄ D ₆ D ₆ | D ₈ D ₁₀ 3 2 | D ₄ D ₆ D ₆ D ₆ | |
| | 10 ⁻⁵ | D ₄ D ₄ D ₆ D ₇ | 4 3 3 3 | D ₃ D ₆ D ₆ D ₆ | 0 0 0 0 |
| | 10 ⁻⁶ | D ₅ D ₆ D ₇ D ₁₀ | 2 2 1 1 | D ₃ D ₄ D ₄ D ₇ | (Normal serum did not neutralize the virus) |
| | 10 ⁻⁷ | D ₁₀ 3 3 3 | 0 0 x x | D ₅ D ₆ D ₆ D ₇ | |
| | 10 ⁻⁸ | D ₈ 2 2 ± | 0 0 0 0 | D ₆ D ₇ D ₁₀ 0 | |
| | 10 ⁻⁹ | 1 1 1 0 | | D ₁₀ 3 3 3 | |
| | 10 ⁻¹⁰ | 1 1 0 0 | | 3 2 2 0 | |
| | 10 ⁻¹¹ | | | 2 ± x x | |
| | 10 ⁻¹² | | | 1 ± 0 0 | |

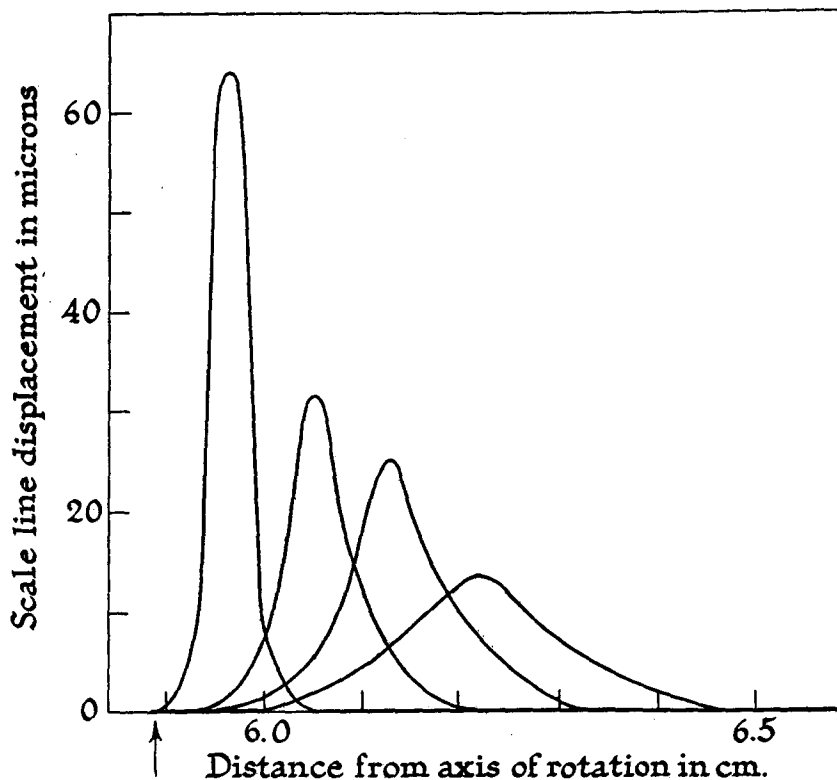
x = mouse died with atypical or no lesions.

The sedimentable protein ranged between 0.01 and 0.07 mg. per ml. in the various experiments, the yield bearing no direct relationship to the age of the culture at least up to 48 hours. The weight of a minimal infectious dose (M.I.D. 50) of the sedimented protein varied from 10⁻¹³ to 10⁻¹⁶ gm.

Determination of the Sedimentation Constant

Appropriately concentrated preparations of the ultracentrifuge sediments obtained from each of experiments II through V were placed in the sector cell

of the analytical ultracentrifuge and movement of the resulting boundaries was observed and measured by both the scale and schlieren methods with the centrifuge operating at 37,500 R.P.M. With the material from experiment II, a very diffuse, poorly defined component appeared almost immediately and moved rapidly out of the field of observation. No proper measurement of the velocity or amount of this component was possible, but it was judged to have



TEXT-FIG. 1. Sedimentation diagram for concentrated influenza virus protein. The four curves represent scale line displacements measured at 5 minute intervals at a centrifuge speed of 18,500 R.P.M.

a sedimentation constant of about $800 \pm 100 \times 10^{-13}$. Only one other boundary appeared, and it was well defined (Text-fig. 1). It showed a moderate amount of spreading as it moved through the cell. In this and in the other experiments, the boundary positions were measured at intervals of 5 minutes for a half hour and the sedimentation constants were found to have the value indicated in Table IV. The sedimentation constants for the three experiments in which the concentration of material was great enough to permit observation

ranged from 20.4 to 31.0. An inspection of Text-fig. 1 shows that for each curve, the standard deviation¹ is roughly 1/3 of the distance the center of the curve is displaced from its starting position, indicated by the arrow. Since the total time of the experiment represented by Text-fig. 1 was less than half an hour, only a small fraction of this spreading could have been due to diffusion. Hence, this spreading is a somewhat exaggerated estimate of the spread in the sedimentation rate of the particle within a given preparation. The standard deviation of the sedimentation constants of the particles in a given preparation is thus about 1/3 the average sedimentation constant for that preparation. The average sedimentation constant of preparation II was 31×10^{-13} . Taking into account the standard deviation of about 1/3 of that value, it can be stated

TABLE IV
Centrifugation Data

| Run | Age of culture | Time after concentration | Concentration of protein | $S_{20} \times 10^{13}$ |
|-----|----------------|--------------------------|--------------------------|--------------------------|
| | <i>hrs.</i> | <i>hrs.</i> | <i>mg./ml.</i> | |
| II | 24 | 1 | 7.00 | 31 and <i>ca.</i> 800 |
| | | 18 | 7.00 | <i>Ca.</i> 800 ± 100 |
| III | 18 | 1 | 0.54 | No boundary |
| | | 18 | 0.54 | " " |
| IV | 24 | 1 | 4.53 | 20.5 |
| | | 18 | 4.53 | 20.4 |
| | | 120 | 4.53 | Completely aggregated |
| V | 48 | 1 | 6.06 | 27.3 |
| | | 18 | 6.06 | 27.2 |

that about 2/3 of the total material in preparation II consisted of particles with sedimentation rate falling within the range 21 to 41×10^{-13} with an average of 31×10^{-13} . The electron microscope studies show that the particles are essentially spherical so that if it is assumed that the particles have a density of 1.35, these figures indicate that two-thirds of the particles of preparation II have diameters between 10 and 15 $m\mu$ with an average of 12.5 $m\mu$. In preparation IV, the corresponding range would be 8.3 to 12.3 $m\mu$ with an average of 10.3 $m\mu$, and in preparation V, 9.6 to 14.2 $m\mu$, with an average of 11.9 $m\mu$. Viewed in this light, the seemingly wide variation in the sedimentation con-

¹The standard deviation of a normal curve is the distance between the maximum ordinate and that ordinate which, with the base line, the maximum ordinate, and a portion of the normal curve, encloses approximately $\frac{1}{3}$ of the total area under the curve.

stants reported in Table IV could be interpreted as being a natural consequence of the normal variation in the size of the particles involved.

It can be seen in Table I that when the influenza virus material was centrifuged $1\frac{1}{2}$ hours at 50,000 g, roughly one-half of the material came out of suspension. When tobacco mosaic virus was sedimented under the same conditions, all of it came out of solution. Hence, the infectious principle of the influenza virus should have a sedimentation constant of not more than 1/2 that of tobacco mosaic virus, or not more than about 90×10^{-13} . It can also be seen in Table I that the infectious material in the influenza preparation is practically completely sedimented in $1\frac{1}{2}$ hours at 90,000 g. Egg albumin spun under identical conditions was sedimented only to a very small extent. Even though the relatively high diffusion constant of egg albumin as compared to that of tobacco mosaic virus makes it impossible to reason quantitatively as above, one can be sure that the sedimentation constant of the influenza material must be many times that of egg albumin or many times 3.5×10^{-13} . In view of the facts, then, that the infectious material seems to have a sedimentation constant many times 3.5×10^{-13} but not more than 90×10^{-13} , the assumption that the infectious material is the reasonably homogeneous protein with a sedimentation constant of from 20 to 31×10^{-13} does not appear unreasonable.

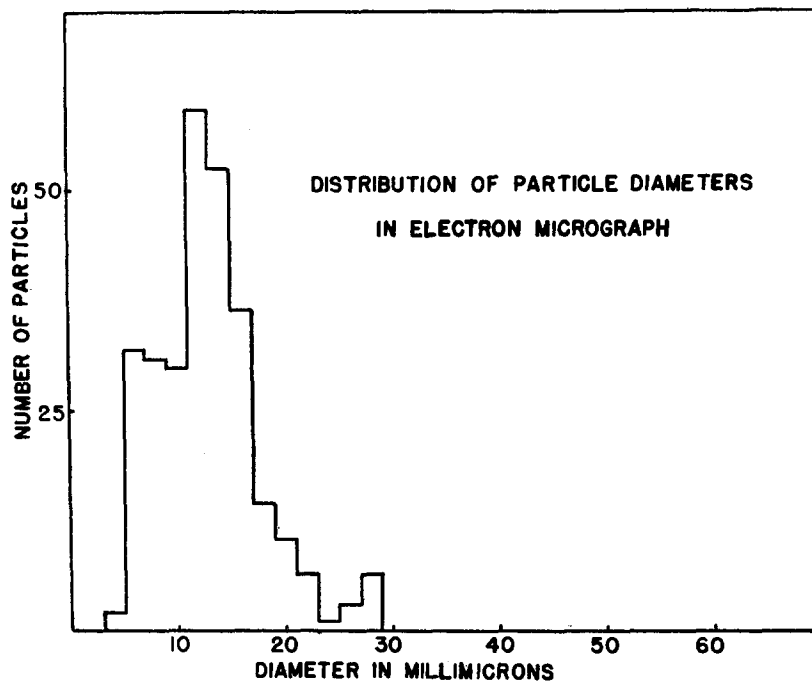
The sedimentation constants determined in the three experiments correspond to those of spherical particles of density 1.35, diameter 10 to 12.5 $m\mu$, and molecular weight of 470,000 to 840,000. The weight of an infectious dose of the sediment from experiment II apparently contained about 300 such particles. In view of the known possibility of aggregation, the inefficient route of inoculation of the mice, and possible neutralizing rôle of non-specific mechanisms in the test animals, infection with as few as 300 to 1000 elementary units is considered an index of a reasonable degree of purity of the virus preparation. This is especially impressive in view of the simplicity of the preparative procedure.

Information as to the nature of the fast moving component ($S_{20} = 800 \pm 100 \times 10^{-13}$) was obtained by repeating the analytical centrifuge runs some hours after the original preparation of the specimens. During the interval of several hours or days the sample was kept at 0°C. As shown in Table IV, the slow moving component completely disappeared during 18 hours of standing in the cold. In experiment IV it was still observable after 18 hours, but was not present after 5 days. In each of these cases the amount of the fast moving component increased with time at the expense of the more disperse material.

Electron Microscopy of the Sedimented Virus

Air-dried preparations of the centrifuge sediments used in the above experiments were observed repeatedly in the electron microscope and the resulting

micrographs, of which Fig. 1 is representative, show none of the large dense bodies seen in filtrates from normal and infected tissue. These micrographs show, however, that the predominating unit is essentially spherical in shape, thus justifying the foregoing estimation of the particle weights from the sedimentation constants. Moreover, all the particles in several such fields were measured, and the distribution of diameters shown in Text-fig. 2 was found. A sharp peak in the curve at a diameter of about 11 $m\mu$ conforms remarkably



TEXT-FIG. 2. Distribution of particle diameters in representative electron micrograph (*cf.* Fig. 1).

well with the previous estimate of the elementary unit from sedimentation data. Furthermore, the degree of spread in particle size observed by this method agrees very well with that estimated from the sedimentation diagram.

Identity of the Virus Used in the Experiments

The identity of our F-12 strain with influenza A virus was checked by mouse neutralization tests, using anti-influenza A swine sera and the IH2 concentrated horse hyperimmune serum prepared by Laidlaw and his coworkers (10) given us through the courtesy of Dr. R. E. Shope of The Rockefeller Institute.

Each of these neutralized at least 1000 to 10,000 lethal doses of our virus preparations, the highest concentrations tested.

DISCUSSION

The previous study of the distribution of the virus of influenza A in extra-embryonic fluids of the infected chick (3) suggested that the elementary infectious unit was considerably smaller than experiments based on lung filtrates had indicated (1, 2). The experiments reported in this paper demonstrate that particles from infected chick embryos which can reasonably be presumed to be the elementary infectious units are essentially spherical bodies having an average diameter of 10 to 12.5 $m\mu$ and a molecular weight of 470,000 to 840,000. The influenza virus seems, therefore, to be one of the smallest pathogenic agents thus far measured, falling in the same size range as that estimated for the viruses of poliomyelitis and foot-and-mouth disease.

Several circumstances have facilitated the isolation and measurement of the influenza virus protein. Among these two are notable: (1) the occurrence in high concentration of the virus in a fluid almost completely free from cellular debris; and (2) the absence from normal embryonic fluids of proteins having sedimentation constants in the same range as that of the virus. Because such interfering substances are absent, it is a relatively simple procedure to isolate the virus material.

The yield of virus protein per milliliter of infected extra-embryonic fluid was such that 50 liters will yield about 1 gm. It is, therefore, evident that enough of the protein can be made available for a detailed analysis.

Many of the viruses pathogenic for animals have been studied under conditions closely resembling those under which earlier measurements of the influenza virus were obtained, *i.e.*, in the presence of cellular elements and debris. At least some of these viruses have been, and others may be, cultured in the developing chick. In the light of the experience with influenza virus a study of the possible occurrence of these other pathogenic agents in egg fluids would appear to be called for. Possibly other estimates of virus size have been obscured by absorption of small units by large tissue particles.

A molecular weight of less than 1,000,000 probably precludes any such structural complexity as that obtaining in the elementary bodies of vaccinia. Preliminary studies have indicated that influenza virus protein virus is no more complicated than tobacco mosaic virus protein, consisting largely of ribonucleoprotein.

SUMMARY

The pathogenic agent of influenza A has been sedimented from infected extra-embryonic fluids of the developing chick embryo by ultracentrifugation. Material so obtained contains two fractions resolvable in the analytical centri-

fuge cell. The first, a homogeneous fraction, showed a sedimentation constant $S_{20} = 20$ to 31×10^{-13} . The second showed a sedimentation constant $S_{20} =$ about $800 \pm 100 \times 10^{-13}$, was much less homogeneous than the first, and was shown to consist principally of aggregated particles of the more disperse fraction. Both fractions contained the virus in essentially equal amounts per unit of protein weight as calculated from nitrogen determinations.

Electron micrographs of the isolated virus protein indicated that the predominating unit is roughly spherical in shape and has a modal particle diameter of about $11 \text{ m}\mu$, in good agreement with the sedimentation data in indicating a molecular weight of about 650,000.

Approximately 300 of the particles having molecular weight of 650,000 were present in the minimal dose producing infection in mice after nasal instillation.

The influenza A virus may now be regarded as one of the smallest pathogenic agents thus far isolated. Preliminary analyses indicate that it is also one of the least complex, being composed principally of nucleoprotein.

It is a pleasure to acknowledge the active assistance and advice of Dr. W. M. Stanley of The Rockefeller Institute for Medical Research, Princeton, New Jersey, during the course of this investigation.

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EXPLANATION OF PLATE 14

FIG. 1. Representative electron micrograph of the influenza virus protein recovered from allantoic fluid by ultracentrifugation. Magnification of electron micrograph 12,500; enlarged photographically to 37,500.



(Chambers *et al.*: Size of influenza A virus)