ELECTRON DENSITOMETRY OF STAINED VIRUS PARTICLES*

By CECIL E. HALL, PH.D.

(From the Department of Biology, Massachusetts Institute of Technology, Cambridge)

PLATES 1 AND 2

(Received for publication, October 4, 1954)

Since biological materials consist mainly of atoms of low atomic number and exhibit only small variations in density, structural inhomogeneities are frequently difficult or impossible to discern by direct observation with the electron microscope. Shadow casting is widely employed for the enhancement of contrast particularly in virus research, but this method is inapplicable to structures embedded in a matrix The enhancement of contrast in depth is achieved through the application of electron stains, which are dense materials that will associate with the structures so as to produce large differences in scattering power between components. Osmic acid (osmium tetroxide) (1) and phosphotungstic acid (PTA) (2) are the most widely used at present. These and other electron stains have been employed for the most part on a purely empirical basis although a few examples exist in which the chemistry of staining reactions has been considered (1, 3–5). In this investigation, quantitative electron densitometry is applied to the problem of measuring the amount of electron stains that can be absorbed by virus particles under various conditions. As test materials, viruses of tomato bushy stunt (BSV) and tobacco mosaic (TMV) were chosen because of their uniformity and because in the past these and similar virus particles have been very resistant to staining methods and difficult to discern under conditions in which shadow casting is inapplicable.

Electron Microdensitometry

Experimentally it has been demonstrated (6, 7) that the focused intensity I at the image plane of an electron microscope is given by the equation

$$I = I_o e^{-Sw} \tag{1}$$

in which I_o is the intensity through an open area, w is the mass per unit area or mass thickness of the specimen, and S is the specific cross section or cross section per gram. Superimposed on the image there will in general be a background intensity I_B caused by electrons scattered beyond the effective aperture of the objective or reflected from the walls of the instrument. Equation (1)

* This investigation was supported by a research grant C-2171 (C2) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

neglects phase contrast effects, holds only for amorphous material and only for areas large compared to the resolution limit. The intensity I is the sum of two fairly distinct components: (1) a component transmitted by the specimen without appreciable change in relative aperture and (2) a component scattered by the specimen but contained within the effective aperture of the objective which is in the order of 10^{-3} to 10^{-2} radian. The scattering cross section S for a given substance will depend on the spherical aberration constant of the lens, the beam potential, and dimensions of a physical aperture if one is present.

Scattering cross sections are not easily measurable with good accuracy but the current data for the instrument used in this work are reproduced in Table I to demonstrate some interesting and significant points. The value for Pd is probably in error on the high side for the same reason that some of the initially

TABLE I

Electron Scattering Cross Sections at 65 Kv.

Substance	Atomic No.	S	σ
		10^4 cm.²/gm.	10^{-18} cm.²
Be	4	3.0	0.45
C	6	3.6	0.72
Al_2O_3	13, 8	3.7	1.0 ave.
SiO	14, 8	3.7	1.4 ave.
Cr	24	3.5	3.0
Ge	32	3.3	3.9
Pd	46	4.5	8.0
Pt	78	3.0	9.8
U	92	3.3	13.
		ave. 3.5	

reported values for metals were too high (6). Otherwise the value of S is nearly independent of atomic number within limits of about ± 10 per cent. The cross section σ per atom increases approximately linearly with atomic number. The figures in Table I are from measurements made through thin evaporated films except for C and Al_2O_3, for which substances the transmission was measured diametrically through spheres of known density. The area involved was small with respect to the cross section of the spheres. It is pertinent to note in this connection that if densitometric measurements are taken close to the edge of a spherical particle of polystyrene latex, the apparent cross section is too large. The reason for this has not been explained. The results in Table I suggest that for a given beam potential and aperture, S is an instrumental constant which could be determined by measuring a standard particle so that absolute values of w could subsequently be found from the ratio I/I_o. Additional data would be desirable before such a procedure could be reliably adopted.

In the absence of numerical values for S, the form of Equation (1) enables us to obtain the ratio of mass thicknesses. For the particular experiments to be described, this requires (1) a calibration graph of the photographic plate showing the optical density D *versus* relative exposure, (2) a measure of the background density D_B, (3) a measure of the optical density D_1 outside of the particle, and (4) a measure of the optical density D_2 at the center of a particle. If I_B, I_1, I_2 are the corresponding intensities, then

$$Sw = \log_e\left(\frac{I_1 - I_B}{I_2 - I_B}\right) \tag{2}$$

in which the substrate thickness has cancelled out. In the absence of numerical values for S and on the assumption that S is independent of atomic number or chemical structure, the ratio of mass thickness for different particles may be found.

In a great part of this and previous densitometric work (6), a calibration graph was constructed for each plate from a series of stepped time exposures in one of the frames of a 2 × 10 inch lantern slide plate. After considerable experience, it was found that for standardized development conditions (4 minutes, *D*-19, 68°F.), the shapes of the curves were sufficiently alike that the curve for one plate could be superimposed on that for another within experimental error if one of the time scales was multiplied by an appropriate constant. This constancy in shape of characteristic curves makes it possible to use a universal graph and dispense with the stepped exposures.

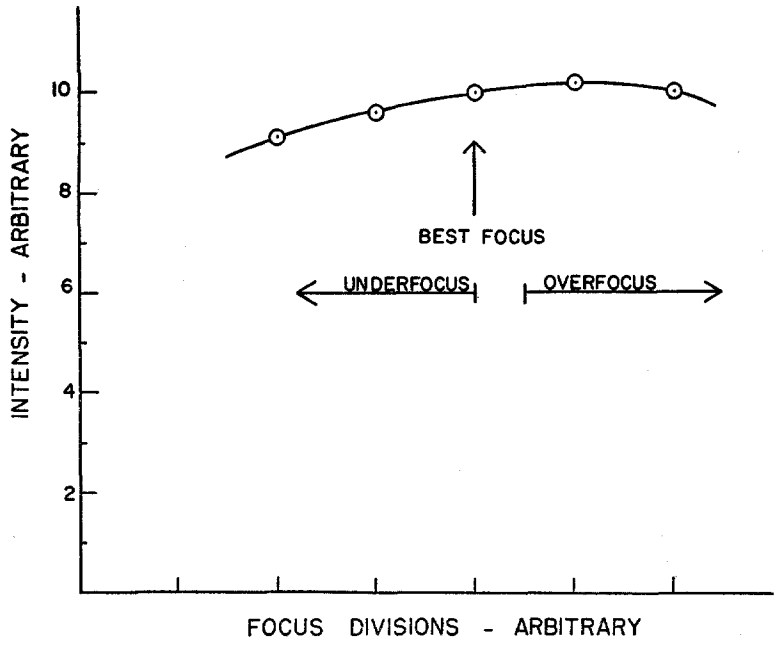
The background density was obtained by including a small portion of a grid wire in each frame. The film must be on the lens side of the grid, otherwise a darkfield intensity will occur inside the shadow of the grid. With the film on the lens side, the density drop at the image of the edge is still not as abrupt as could be desired because the edge is slightly out of focus. For this reason, the background density was measured at an arbitrary distance of about 1000 A inside the shadow.

Density measurements were made on a microphotometer which projected a magnified image of the plate on a screen where an aperture small compared to the particle size passed light from selected areas to a light-sensitive device. Densities D_1 were measured at the center of particles and densities D_2 were measured at three or four positions around the particle. All plates were recorded as a through-focus series at a magnification of 20,000 and measurements were made on the frame closest to true focus on the underfocused side. This procedure is necessary since the scattered (or darkfield) intensity being of greater aperture is rapidly defocused as the lens current deviates from the true setting. Text-fig. 1 shows the intensity in arbitrary units measured at the center of a stained particle of BSV as a function of the fine focus divisions that were used in recording a through-focus series. At best focus, the intensity is close to a

maximum and the contrast of the particle is low. For uniformity of optical conditions, no physical aperture was used in the objective lens.

Measurements of Electron Stain Absorption

Under the conditions of these experiments (65 kv., no objective aperture), the unstained, air-dried BSV particles were very difficult to discern in electron micrographs. Values of *Sw* calculated from such micrographs were lower than would be expected for a spherical particle of the accepted mass of BSV (8).



TEXT-FIG. 1. Graph showing the variation with focus of the relative electron image intensity at the center of a stained BSV particle.

The discrepancy can be accounted for by assuming that the particles are flattened during drying so as to decrease their vertical diameter by about 25 per cent. Conventional stains such as buffered PTA in pH range 4–7 or OsO_4 near neutrality produced very little increase in contrast as compared qualitatively with their effects on other biological materials. As conditions and stains were varied in an effort to increase the amount of stain absorption, the value of quantitative densitometry became apparent. A visual evaluation of micrographs can often be misleading owing to the fact that appearance to the eye is governed by the level of exposure, photographic contrast, thickness of support-

ing film, and other factors. In a series of tests with unbuffered 12 PTA in increasing concentration and consequent decreasing pH, no large amount of stain was taken up until the pH was below about 2. Since BSV is stable only above pH 2 (9), this indicates that the conditions necessary for efficient staining are destructive. Some of the results are shown in Table II where in the third column the ratios of *Sw* for stained over unstained particles are tabulated. Each ratio given is the average of measurements from at least two independent plates with 10 particles measured on each plate. Consistency of measurements between individual particles and between separate plates was good. Since unstained particles are undoubtedly flattened and broadened when they are air-dried, the ratios in the third column would be smaller probably by about 20 per cent if the comparison were made with undistorted, unstained particles prepared for example by freeze-drying. In these and other experiments, the reaction reached a maximum in a minute and all tabulated results unless other-

TABLE II

Electron Stains on BSV

Reagents	pH (unbuffered)	*Sw* relative to unstained air-dried particle	Average diameter
			Å
1 per cent 12 PTA	1.8	2.6	290
1 per cent 12 PTA 10 min.	1.8	2.7	320
10 per cent 12 PTA	1.3	3.4	300
40 per cent 12 PTA	0.7	3.5	285
53 per cent 12 PTA	0.6	3.5	320

wise noted are for 1 minute staining time. Higher concentrations than those listed in Table II were used but no significant increase in stain absorption was achieved. The maximum effect for 12 PTA occurs at pH about 1 and results in a mass thickness 3.5 times that for the unstained air-dried particles.

Although the usefulness of PTA as an electron stain has been established on an empirical basis, hypothetically the large number of tungsten atoms per molecule might be expected to result in a very high efficiency compared to reagents of lower molecular weight. Actually, the efficiency of stains used on BSV is not in proportion to the molecular weight as can be seen in Table III where results are shown for a variety of reagents. Except for OsO_4 which was in veronal buffer, these stains are unbuffered. The first four reagents listed containing 12 to 24 atoms of tungsten per molecule are significantly more efficient than $PtCl_4$ and $Th(NO_3)_4$ with only one heavy atom per molecule, but the difference is small compared to the differences in molecular weight. $LaCl_3$ and OsO_4 at pH near neutrality were absorbed in relatively small amounts compared to the others. If the pH of these two reagents is reduced in an effort to increase absorp-

tion, the virus particles show signs of disintegration or may be destroyed completely.

Note in connection with Table III and similar tabulations that the ratios are proportional to the mass of stain absorbed plus the mass of the unstained particle. The relative amount of stain taken up is obtained by subtracting unity from the ratios. On this basis it is seen that 10 per cent $PtCl_4$ is more than twice as efficient as buffered 1 per cent OsO_4, and that 40 per cent 24 PTA is

TABLE III

Electron Stains on BSV

Reagents	pH (unbuffered except OsO_4)	S_w relative to unstained air-dried particle	Average diameter
			A
40 per cent 12 PTA	0.7	3.5	285
40 per cent 20 PTA	0.7	3.2	310
40 per cent 24 PTA	1.1	3.6	295
40 per cent SiTA	0.94	3.2	285
10 per cent $PtCl_4$	0.86	2.5	295
30 per cent $Th(NO_3)_4$	1.4	2.4	250*
7.5 per cent $LaCl_3$	5.9	1.9	300
1 per cent OsO_4	7.5	1.7	315

* Particles partially disintegrating.

TABLE IV

Electron Stains on BSV

Reagents	pH (unbuffered)	S_w relative to unstained air-dried particle	Average diameter
			A
40 per cent 12 PTA → 30 per cent $Th(NO_3)_4$	0.7 → 1.4	4.1	320
40 per cent 12 PTA → 10 per cent $PtCl_4$	0.7 → 0.86	3.9	320
40 per cent 12 PTA, 10 per cent $PtCl_4$	1.0	4.0	320
7.5 per cent $LaCl_3$ → 40 per cent 12 PTA	5.9 → 0.7	3.8	275

four times as efficient as buffered OsO_4. Particles treated with 30 per cent $Th(NO_3)_4$ have an abnormally small diameter and are disintegrating.

In Table IV are listed the results obtained when BSV was stained with two reagents together or in succession. An arrow indicates that the reagents were applied in succession with a rinse in between. Duration of the application was always 1 minute. When positive metallic ions were used in conjunction with PTA, significantly greater stain absorption was achieved over what would result with PTA alone. The increase in mass thickness, however, is only one-third the amount that these same positive ions produced when they were used

alone as may be seen by comparing Tables III and IV. Micrographs of BSV stained with the last two reagents in Table IV are reproduced in Figs. 1 and 2. Fig. 1 represents about the highest density of stain that could be achieved and the electron intensity within the image of the particles is 70 per cent of the intensity outside.

All the stains discussed above except $LaCl_3$ and OsO_4 represent concentrations and pH's which are probably far too drastic to be recommended for most biological materials. Furthermore, the excessive stain absorption that was sought here need not be necessary for most purposes. In general we may conclude that to increase the amount of stain absorbed, we must go toward conditions which would normally be regarded as destructive but precipitating action must be such that morphology is preserved within resolvable dimensions. If, for example, the pH of 12 PTA in concentrations of 1 per cent is reduced to about 1 with HCl, BSV particles show evidence of disintegration and the residue

TABLE V

Electron Stains on BSV

Reagents	pH	S_W relative to unstained air-dried particle	Average diameter
			Å
1 per cent 12 PTA → 1 per cent 12 PTA	7.0 → 0.7	3.6	290
1 per cent OsO_4 → 1 per cent 12 PTA	7.5 → 0.7	3.2	280
1 per cent 12 PTA → 1 per cent 12 PTA	1.8 → 0.7	2.7	290

contains much less stain than would have been taken up if the same pH had been reached by increasing the PTA concentration. The lowering of pH with HCl can result in greater stain absorption but this may be accompanied by partial disintegration of the structure. In order to achieve maximum stain absorption with optimum preservation of morphology, we should attempt to fix and stain the more unstable parts of the structure before proceeding to drastic conditions that may be necessary for the more refractory components. Experiments bearing on this problem are listed in Table V. Here the BSV was treated with three relatively mild reagents before being exposed to 1 per cent 12 PTA with HCl at pH 0.7 which by itself is morphologically destructive. The first combination in Table V consisting of pretreatment with 1 per cent 12 PTA at pH 7.0 preserves morphology well (Fig. 3) and the final density of particles is about the same as could be produced by the application of PTA in extremely high concentration. The edges of the particles are less sharply defined, however, than those which have been stained at high concentrations to comparable mass thickness such as, for example, Figs. 1 and 2. The second combination in Table V consisting of pretreatment with OsO_4 at pH close to

neutrality similarly demonstrates a stabilization against the final drastic condition although the final density of the particles is significantly less than was achieved with PTA pretreatment. Morphology was well preserved. The last example in Table V which shows the results for pretreatment with unbuffered 1 per cent PTA at pH 1.8 indicates that although the pretreatment produces appreciable stabilization, the initial conditions were too drastic since the total amount of stain absorbed is only 65 per cent of that for pretreatment with PTA at neutrality.

In the last column of Tables II through V, a figure is given for the average diameter which was obtained from twenty measurements taken at random from two different plates. These figures are included for completeness although their significance requires qualification. Diameters of single particles cannot be measured to better than about 15 A and most of them do not appear to be perfectly round. The calibration of the microscope is uncertain to ± 3 per cent. There is some variation in diameter within the same field and apparent diameters of isolated particles are generally greater than the diameters of particles that are packed together. This effect can be seen in Fig. 1 by comparing the particles in the packed group to the left with particles that are isolated. These variations are undoubtedly introduced by the conditions of staining. Average diameters for all reagents listed in Tables II through V, excluding $Th(NO_3)_4$ which is obviously destructive, range from 275 to 320 A and the average of all measurements is 300 A. The difference between values near the extremes is considered to be significant and must reflect in these cases a small amount of swelling or shrinking during reactions. However, for more than half of the reagents listed, including many having close to the maximum absorption of stain, measured diameters are within experimental error of an acceptable diameter for BSV (10).

Like BSV, TMV is stable over a wide pH range reported as pH 1.5–8.5 (9). TMV absorbs very little stain in this range but can be stained heavily at lower pH with the same reagents which have a high efficiency on BSV. Because of low contrast, it is difficult to obtain reliable measurements on unstained TMV for the purpose of estimating the relative increase in mass. Such measurements as could be made, however, indicated that the maximum ratio of Sw for stained over unstained particles is about 2, which is lower than for BSV. This may be connected with the internal hydration of TMV which is very low or zero. A micrograph of heavily stained TMV is reproduced in Fig. 4.

Estimates of Mass

In Tables II to V, the ratio of Sw for stained over unstained particles has been used as the most convenient and appropriate measure of staining efficiency since results are of relative significance without assumptions regarding numerical values of S. It is interesting, however, to calculate absolute densities and masses on the assumption that for this instrument S is 3.5×10^4

cm.²/gm. as indicated in Table I. For 40 per cent 12 PTA, the ratio is 3.5, which corresponds to $Sw = 0.33$. (In general, Sw can be obtained for any of the particles in Tables II through V by multiplying the ratio in the third column by 0.095.) If ρ is the density of the particles and D is their thickness normal to the film (285 A), $S\rho D = 0.33$ and $\rho = 3.3$ gm./cm.³ Such a high density indicates that the particles must have physical properties approaching those of inorganic metallic compounds and strength to resist distorting forces such as the force of surface tension during drying. In highly stained specimens observed with the plane of the film at 45° to the electron beam, there is no evidence of an asymmetrical particle shape as would be expected if the particles were flattened discs.

The high density of stained BSV particles raises a question as to the space available within the particle for deposition of reagents. Excluding water of hydration, the mass of a 12 PTA molecule is 97.5 per cent contained in 12 WO_3 groups. We assume for purposes of estimation that the density is close to the density of WO_3 which is 7.2. For example, particles stained with 40 per cent PTA have a calculated density of 3.3. If we assume that the stain is attached to a nucleoprotein framework which has a density of 1.35, a volume equal to 33 per cent of the whole particle would have to be filled with WO_3 in order to produce the average density of 3.3. Space available for such a deposit could be that produced by extraction of material during staining or that occupied originally by internal water of hydration. BSV is known to be highly hydrated but estimates of the ratio of internal to external hydration can vary widely according to particular physical constants that are chosen for the calculation. Leonard *et al.* (10), using x-ray data and a molecular weight of 9.3×10^6 given by Williams and Backus (8), calculate internal hydration of 0.27 gm. of water per gm. of virus and an external hydration of 0.50 gm. of water per gm. of virus. These figures would indicate that very little material need be extracted to provide the necessary volume for stain. However, the fact that TMV with very little, if any, internal hydration can take up stain equal to its own weight without measurable increase in width indicates that about 20 per cent of the volume must be extracted to provide space for the stain.

Lanthanum ions react with nucleic acid (4, 11) and Caspersson *et al.* (11) state that these ions do not react with proteins on the acid side of their isoelectric point. At pH 5.9 (Tables III and IV) which is above the isoelectric point for BSV, $LaCl_3$ is probably not specific for nucleic acid since the increase in mass corresponds to about 14 atoms of La per atom of P in the BSV.

Anomalous Images

In the course of this investigation, several anomalies were observed which have a bearing on the interpretation of images from stained specimens. Four examples of such anomalous images are reproduced in Figs. 5 to 8. Fig. 5 shows BSV stained with freshly prepared 10 per cent $AgNO_3$. Each BSV particle

has associated with it 6 to 12 dense particles, presumably silver, whose diameters are mostly in the range 25 to 50 A. The contrast of the nucleoprotein is so low that the outline of the BSV particles cannot be distinguished. The stain particles produce no consistent pattern that might reflect structure in the BSV. Since this stain, when used on other materials such as collagen or TMV, also produces similar random particulates, we conclude that the particles seen in Fig. 5 represent adsorbed colloidal particles rather than stained subunits of the virus structure, though there remains the possibility that there is some specificity in the attachment.

Another anomaly is shown in Fig. 6 which is a micrograph of BSV stained heavily with 40 per cent 24 PTA. Many but not all of the particles have a bright center which in a through-focus series follows the characteristic sequences of intensity that would result if there was a small region with very low scattering power and about 75 A diameter in the center of the particles. Since this appearance has been observed only with excessively high concentrations of PTA, it is interpreted to mean that the stain has not been able to penetrate uniformly to the center of the particles owing to the heavy precipitate in the outer part.

In Fig. 7 is reproduced a micrograph showing a close packed monolayer of BSV particles on a collodion film. The BSV is very lightly stained but stain is lodged in the interstices to produce an appearance like that of a honeycomb. This specimen was treated with 5 per cent PTA at pH 4.6 at which high pH stain absorption is almost negligible. The PTA does, however, penetrate between the particles and is not all removed by routine washing. Particles as lightly stained as these can be very greatly distorted on drying as can be seen in Fig. 7 particularly around the edges of the holes in the monolayer. It might be suggested that there is a stain-receptive shell around the BSV particles which would result in an appearance like that of Fig. 7. No such structure has, however, been observed with isolated, well washed particles.

Fig. 8 shows BSV particles which were treated with 5 per cent PTA at pH 4.6 and purposely given a wash insufficient to remove all of the reagent. The lightly stained particles are embedded in the dried reagent and therefore appear as holes because of their relatively low scattering power. Also to be seen are filaments of unknown origin showing as light streaks against a dark background. The smallest of these filaments measures about 40 A across. Although the effect shown in Fig. 8 is the opposite to what is usually sought by the use of electron stains, the visibility of particles of low scattering power can be enhanced as well, if not better, by surrounding them with dense material rather than impregnating them with dense material.

SUMMARY

Methods are described for determining the relative mass of particles in electron microscope specimens through the measurement of photographic

densities in recorded images. These methods were applied to a quantitative study of the amounts of electron stains that could be associated with the particles of tomato bushy stunt virus (BSV) and tobacco mosaic virus (TMV). In the pH range above 2 where the viruses are stable, the amount of stain absorbed is too small to produce adequate contrast in the electron microscope. Maximum stain absorption was achieved at pH about 1 where with several reagents and combinations of reagents the mass of BSV could be increased to about four times that of the unstained particles. Optimum results were obtained with phosphotungstic acid alone or in combination with Pt, Th, or La ions. Since the pH conditions for high stain absorption are normally destructive, morphology is satisfactorily preserved only when the phosphotungstic acid is applied in concentrations of 10 per cent or greater or when the use of destructive reagents is preceded by a preliminary fixation under mild conditions. Maximum staining of TMV increased the mass of the particles to about two times that of the unstained.

Estimates of the mass of heavily stained BSV particles indicate that their density is 3.3 gm./cm.[3] The high internal hydration of BSV probably accounts for the greater stain absorption and penetration compared to those of TMV which has very low or zero internal hydration.

Anomalous images resulting from the use of electron stains are shown and discussed.

The author wishes to express his appreciation to Professor R. C. Williams who provided the virus samples used and to Mrs. B. M. Ingber and Mrs. J. D. Grosser for their technical assistance in this work.

BIBLIOGRAPHY

1. Porter, K. R., and Kallman, F., *Exp. Cell Research*, 1953, **4**, 127.
2. Hall, C. E., Jakus, M. A., and Schmitt, F. O., *J. Appl. Physics*, 1945, **16**, 459. 1945, **16**, 459.
3. Lamb, W. G. P., Stuart-Webb, J., Bell, L. G., Bovey, R., and Danielli, J. F., *Exp. Cell Research*, 1953, **4**, 159.
4. Calvert, F., Siegel, B. M., and Stern, K. G., *Nature*, 1948, **162**, 305.
5. Kahler, H., and Lloyd, B. J., Jr., *J. Nat. Cancer Inst.*, 1952, **12**, 1167.
6. Hall, C. E., *J. Appl. Physics*, 1951, **22**, 655.
7. Ellis, S. G., and Hillier, J., Congrès de Microscopie Electronique, Editions de la *Revue d' Optique*, Paris, 1952, 79.
8. Williams, R. C., and Backus, R. C., *J. Am. Chem. Soc.*, 1949, **71**, 4052.
9. Bawden, F. C., Plant Viruses and Virus Diseases, Waltham, Chronica Botanica Company, 1952.
10. Leonard, B. R., Jr., Anderegg, J. W., Shulman, S., Kaesberg, P., and Beeman, W. W., *Biochim. et Biophysic. Acta*, 1953, **12**, 499.
11. Caspersson, T., Hammarsten, E., and Hammarsten, H., *Tr. Faraday Soc.*, 1953, **31**, 367.

EXPLANATION OF PLATES

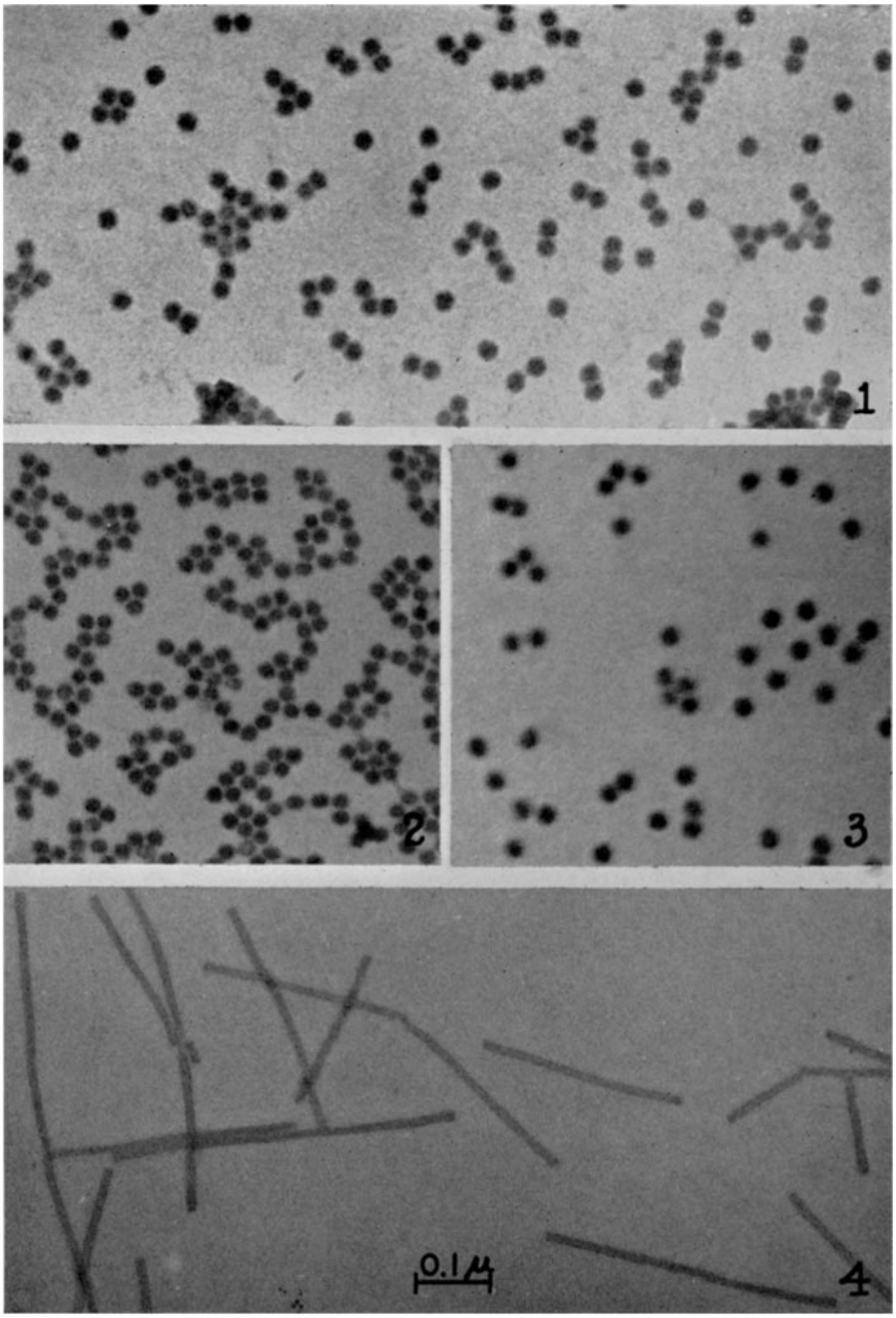
PLATE 1

FIG. 1. BSV particles stained with a solution at pH 1 containing 40 per cent 12 PTA and 10 per cent $PtCl_4$. × 95,000.

FIG. 2. BSV particles stained with 7.5 per cent $LaCl_3$ at pH 5.9 followed by 40 per cent 12 PTA at pH 0.7. × 95,000.

FIG. 3. BSV particles stained with 1 per cent 12 PTA buffered to pH 7.0 followed by 1 per cent 12 PTA in HCl at pH 0.7. × 95,000.

FIG. 4. TMV particles stained with 40 per cent 24 PTA at pH 1. × 95,000.



(Hall: Electron densitometry of stained virus particles)

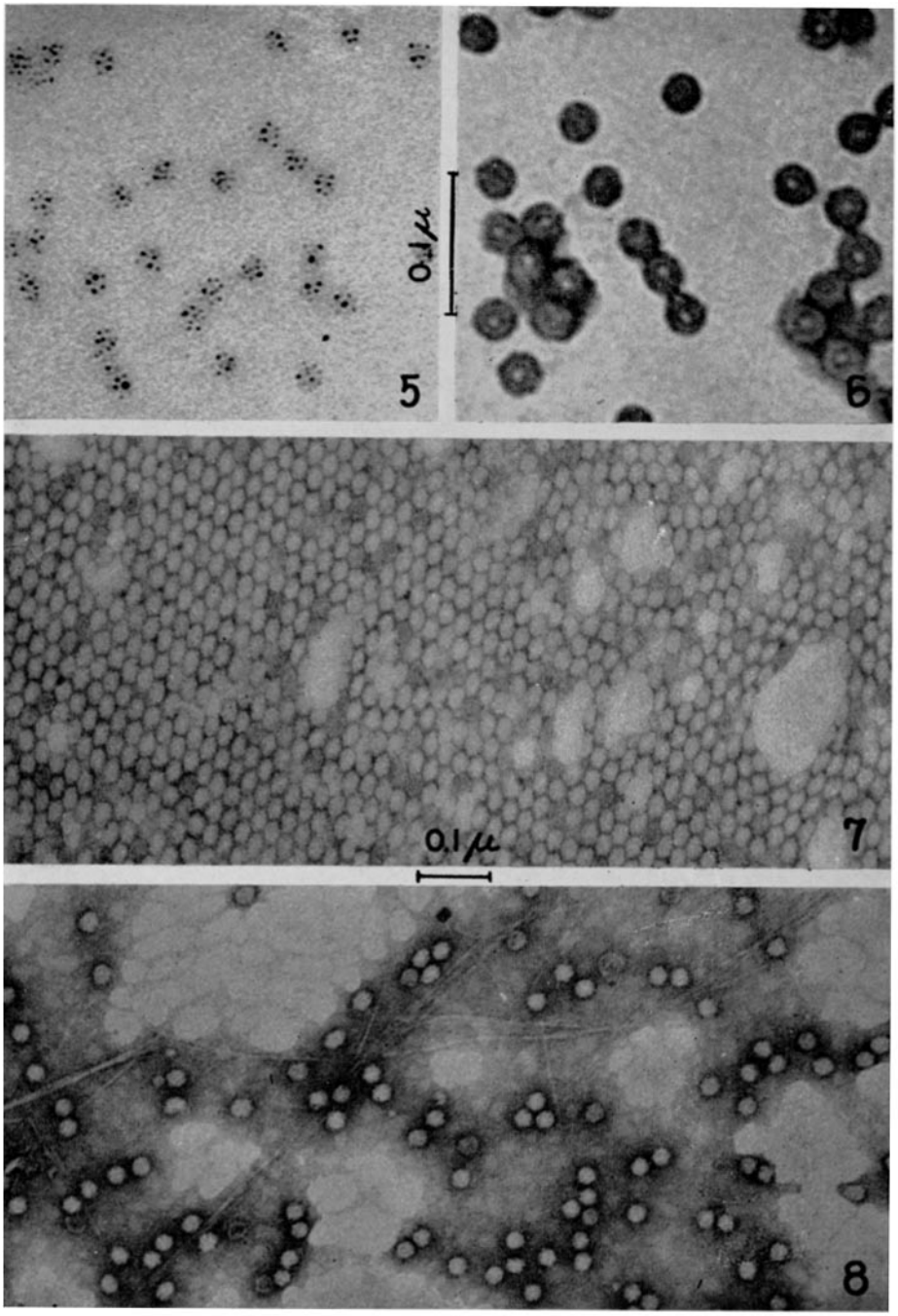
PLATE 2

FIG. 5. BSV particles treated with 10 per cent $AgNO_3$. × 190,000.

FIG. 6. BSV particles stained with 40 per cent 24 PTA at pH 1 showing particles with hollow centers attributed to insufficient penetration of stain. × 190,000.

FIG. 7. BSV stained very lightly with 5 per cent 12 PTA at pH 5.6 but with stain lodged between particles. × 95,000.

FIG. 8. BSV stained very lightly with 5 per cent PTA at pH 4.6 and insufficiently washed. × 95,000.



(Hall: Electron densitometry of stained virus particles)