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ELECTRON-MICROSCOPY OF VIRUSES: I. STATE OF AGGREGATION OF TOBACCO MOSAIC VIRUS.

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BAWDEN and Pirie (1937) noted that the properties of purified preparations of tobacco mosaic virus differed in a number of ways from those of virus as it occurs in freshly extracted sap. They suggested that virus particles were aggregating linearly during the course of purification, and that the greatly elongated rods characteristic of purified preparations might be artefacts. Since then evidence that purified perparations are heterogeneous in particle size and that preparations made by different methods may contain particles of very diverse lengths has come from studies of various physical properties. Such estimates of the length of virus particles in purified preparations have ranged from less than 300 mμ. to more than 1400 mμ. (*cf.* review by Pirie, 1945). The electron microscope provides an attractive direct* method of measuring particle size, and has already been used by several workers. It has confirmed that particles are more or less uniform in width and vary considerably in length, but there has been much difference of opinion as to the size of the basic units that aggregate to produce the long rods.

* In fact it is little more " direct " to use a lens to combine a diffraction pattern into an image which is then measured, than to record and measure the diffraction pattern itself. However, it is more straightforward to relate two lengths, that of the image to that of the object by a simple ratio (the magnification), than to deduce a length from some other physical quantity of more complicated dimensions. It is with these considerations in mind that the word " direct " is used here.

Kausche, Pfankuch and Ruska (1939) made the first measurements and gave a length of 150 to 300 mμ. Melchers, Schramm, Trurnit and Friedrich-Freska (1940) reported lengths of 140 and 190 mμ. Schramm (1943) also refers to electron micrograms* of tobacco mosaic virus as showing particle lengths of "about 200 mμ." Stanley and Anderson (1941) concluded from " measurements of the lengths of all of the 58 particles in two selected fields " that a unit having a length of 280 mμ. predominated. They concluded that the difference between their measurements and those of Melchers *et al.* was due to a difference in the virus strains. Lauffer (1944), from measurements on two prints from two preparations, concluded that the particle length was approximately 270 mμ., but that particles of approximately double this length were also present. However, Frampton (1942) and Rawlins (1942) did not consider the conclusions of Stanley and Anderson valid. Frampton, from measurements of the published electron-micrograms of Stanley and Anderson, concluded that they showed the particles to be made up by the aggregation of units 37 mμ. long. Rawlins did not admit even this regularity, and on the basis of new measurements on the same micrograms, correctly maintained that there are no statistically significant regularities at all. However, in a later publication, Rawlins, Roberts and Utrech (1946) claim that the majority of particles are about 300 mμ. in length, that longer particles are formed by aggregation of these, and that smaller units are due to breaking of rods during the drying which is necessary before examination in the electron microscope.

Light has recently been shed upon this somewhat chaotic situation by the demonstration (Bawden and Pirie, 1945) that it is possible to prepare tobacco mosaic virus in different states of aggregation, and that the particle length of any given preparation is largely a function of its previous treatment. They arrived at their conclusions on the basis of serological, ultracentrifugation and birefringence data, and their conclusions are fully confirmed by the electron microscope data presented in this paper.

EXPERIMENTAL.

Material.

Bawden and Pirie (1945) found that the conditions obtaining in expressed sap led to aggregation of tobacco mosaic virus particles. By using suitable methods, involving freezing and washing the leaves before mincing, they were able to extract virus while causing little aggregation. The virus in such extracts, however, was still far from homogeneous, and could be separated by ultracentrifugation into fractions with different properties. There is a steady gradation in properties from one fraction to another, but their method separates four fractions. One (D) sediments and compacts at 17,000 r.p.m. (R.C.F. 17,000), another (A) sediments but does not compact at 40,000 r.p.m. (R.C.F. 80,000), whilst B and C are intermediate. Fractionations were made of material which had been extracted by mincing and also from material subsequently passed through a triple-roller mill. These will be referred to as mincer and mill extracts respectively. We received for electron microscope examination various fractions prepared from leaves of tobacco (var. White Burley) infected with a mild strain of

* Chilton (1944) has suggested that it is convenient to restrict the terminations " -scope," " -graph," and " -gram " to the viewing instrument, the recording instrument, and the record respectively, as is usual for spectroscope, spectrograph and spectrogram. This terminology is adhered to here so that the more usual " electron-micrograph " becomes " electron-microgram."

tobacco mosaic virus, and also some of these same fractions after they had been subjected to various treatments. Purified preparations of potato X (Bawden and Pirie, 1938*a*), tomato bushy stunt (Bawden and Pirie, 1938*b*) and tobacco necrosis (potato culture) viruses (Bawden and Pirie, 1942) were available for comparison. The same tobacco mosaic strain and potato virus X were also examined in sap from tobacco plants (var. White Burley) infected with these viruses. The sap was prepared by freezing and centrifuging to remove chromoproteins.

Methods.

Specimens for comparison with each other were prepared all at one time and as soon as received to obviate as far as possible changes which may occur in the test-tube. A drop of dilute solution was placed on a membrane-covered metal grid and was rapidly dried in a vacuum desiccator. Because of drying and edge effects (see p. 337) the drops were usually placed asymmetrically so that the edge of the drop crossed the area to be examined in the microscope. Such drops were flat and about 2 mm. in diameter. Rather larger symmetrically placed drops were also employed, and microdrops covering an area 20–50μ in diameter have been examined. Concentration of virus in the suspension was varied from 1 in 10^4 to 1 in 10^6, according to the expected particle size, and higher concentrations were used for microdrops.

The membranes used in this work have been, in most cases, collodion stripped from glass. In our experience it is impossible to make really satisfactory membranes by spreading on water. When such membranes have once been in the microscope or have been kept in either a dry or a damp atmosphere for some days they can no longer be exposed to the beam without considerable risk of disintegration. Stripping from glass produces much more uniform and robust membranes, which can be stored indefinitely without any special precautions other than to keep them dust-free and can be placed in the microscope a number of times. Microscope slides (3 in. $\times$ 1 in.), selected for even surface, are prepared by boiling in cleaning fluid, washing under the tap and then in grease-free distilled water and draining. They are then polished with MgO, moistened with alcohol on a cotton-wool swab, the MgO removed with a clean dry cotton-wool swab, and finally by washing in dilute HCl followed by distilled water. The water is replaced by alcohol, which is finally removed by flaming. On such a clean and polished surface both aqueous and organic solutions will spread with ease, and from it membranes are readily removed.

Collodion (Schering celloidin is satisfactory) is dissolved in analar amyl acetate. 0·25 per cent solutions are most generally satisfactory. One surface of a prepared slide is flooded with solution, the surplus is drained off and the slide is stood on end on filter-paper in a desiccator at atmospheric pressure until the solvent has evaporated. Drying in a desiccator is useful to prevent " blooming " of the film. Slides bearing dried membranes can be stored indefinitely in Coplin jars at this stage. The membrane thus produced is wedge-shaped and is approximately 10 mμ. thick about the middle of the slide and, provided the glass surface was good, it is free from holes. The film is divided into $\frac{1}{2}$ cm. squares by scoring with a needle and the slide is lowered gently into a dish of clean, grease-free distilled water at an angle of approximately 30° to the horizontal. The squares of membrane become detached and float on the surface.

The water surface is best prepared by filling a large crystallizing dish so full that the water meniscus stands above the brim, and then sweeping the excess off with a clean glass strip held in contact with the rim. In cold weather it may be advisable to heat the water to about 18° C. A mounting grid is placed on each square, and both are removed and dried and the specimen placed in the usual way.

Five to six specimens were made from each virus preparation and five to ten micrograms taken at random intervals over each specimen, no attempt being made to select the fields other than to try to ensure that virus was being recorded each time.

The electron microscope used was the R.C.A. Type EMB2. Most observations and photographs were made with accelerating potentials of 60kV, since Crook, Sheffield and Chilton (1945) have shown that higher contrast is obtained in the negatives at higher kilovoltages when tenuous specimens are being examined. Earlier observations before this information was available were made at 45 kV. The photographic plates used were the special Contrasty Lantern and Photomechanical of Messrs. Ilford Ltd., the latter being preferred. These were developed with rocking or brushing for 2½ mins. in Ilford ID16 and 2 mins. in Ilford ID13 developers respectively. The temperature of development was 18° C. We have found it advisable to aim at fairly dense negatives (density 1 to 2) in order to realize the maximum available contrast of the plates. Crook *et al.*(1945) have shown that whereas with light, the characteristic curves of the emulsions used here have a fairly sharp " foot " and begin to rise steeply from densities below 0·5, with electrons the " foot " is much more extended, and the curve does not reach its maximum slope until densities of 1·0 or more are reached.

For magnification calibration a diffraction grating replica has been used as primary standard. Earlier methods of calibration (R.C.A. Handbook, 1940) have used the light microscope and a stage micrometer as primary standard, and were limited by the necessity of having a small standard object, of assuming that the magnification of standard object and stage micrometer were identical, and by the necessity of having a good travelling microscope in order to measure the small light images with sufficient accuracy. The resolving power of the light microscope was also a limiting factor. Burton, Barnes and Rochow (1942) proposed the use of diffraction grating replicas as standard objects. They stated that shrinkage was constant and amounted only to 1½ per cent. However, in our experience, shrinkage or stretching may occur and either may vary in amount up to 1½ per cent. The alternative was to use the grating only as a mould, and measure the spacing on the actual replica. No first grade travelling microscope was available for this purpose, but we have been able to devise an optical method, using very simple apparatus.

The diffraction grating was a 14,400 lines/inch concave nickel grating kindly placed at our disposal by the National Physical Laboratory. We have found that the most suitable method of cleaning is to swab gently with a Na lauryl sulphate solution, wash in distilled water followed by alcohol and to dry in a desiccator. Replicas were made by flooding the grating with 0·5 per cent formvar solution in water-free dioxan, and after evaporation of the solvent, stripping on to water as described above. The replicas were transferred to the highly polished side of copper grids of 200/inch mesh in the usual way. The grids were mounted in a holder on a student's type spectrometer and adjusted in the usual

way (Watson, 1930). If no Gaussian eyepiece is available, a substitute can easily be made by supporting a microscope cover-glass at an angle of 45° over the normal eyepiece with a piece of soft wax. The light source was an 80 watt Hg-arc (G.E.C. " Osira " electric discharge lamp with the outer bulb removed), although a higher power would have been better. The arc was focused on the slit of the spectrometer with a short focal length lens—a substage condenser (Leitz aplanatic N.A.1.4) with the top lens removed worked very well—so that it just covered the slit. Sufficient light was thus obtained for the collimated beam to be stopped down to 1/2 mm. diameter and still yield visible 1st and 2nd order images of the Hg green and yellow lines, provided the room was dark. The beam was conveniently stopped down by supporting with a clamp the iris diaphragm from a microscope substage assembly. Measurements of the diffraction angles of the Hg green and yellow lines thus gave directly the average replica spacing over the area covered by the R.C.A. type B2 microscope. Other types which have a larger field would not restrict the lighting conditions so much. In practice it was found that the diffracted images were not single, but were a narrow pattern of lines due to variation in replica line spacing and to slight buckles in the surface of the grid. However, in the replicas selected for use, the total width of this pattern corresponded to much less than 1 per cent of the average grating spacing.

As with other standard objects, its comparatively large size necessitated the taking of the initial calibration pictures with the object in the " low magnification " position. For internal calibration, using this initial picture as standard, we have found diatom fragments to be most suitable. For the step across from the " low " to " high magnification " positions fragments were selected which had holes just discernible in the " low " position, and for internal calibration in the " high " position those with holes less than 0·1μ diameter in convenient positions. The centres of the images of these holes on the negative formed convenient points on which to set the cross-wires of a travelling microscope. Suitable fragments are easily obtained by shaking up a little kieselguhr in water and standing overnight. The very fine particles are removed by pouring off the remaining suspension. The sediment is again shaken up with water, and after standing two or three hours yields a suspension of particles of a convenient size. It can be poured off and kept, the addition of a drop of chloroform discouraging bacterial contamination.

As this paper deals only with the qualitative aspects of the problem, and as lens aberrations make different corrections necessary for particles in different positions, no attempt has been made to give exact magnification data for each microgram. The figures given are therefore only approximate (accuracy $\pm$ 20 per cent). In a later paper quantitative data will be presented in detail and the necessary corrections discussed.

RESULTS.

Bawden and Pirie (1945) have shown that, passing from their A to their D preparations, there is a continual gradation in properties. The sedimentation constant increases, the double refraction of flow increases, the rate of precipitation with antiserum increases and the tendency to form a zone of antigen excess decreases. At the same time the character of the precipitate changes from

that typical of somatic to that typical of flagellar antigens. As they have indicated, this can be most satisfactorily interpreted as an increase of particle length.

Fig. 3 to 6 are electron micrograms of a set of preparations from mill extract. They show that there is a considerable increase in particle length on passing from one end of the series to the other. Fig. 3 is a typical field from an A preparation. Here no rods can be distinguished, although a few particles may be asymmetric. Indeed, so untypical of tobacco mosaic virus as it is generally recognized are micrograms of A-type preparations, that it is impossible to distinguish them at a glance from tomato bushy stunt and tobacco necrosis viruses, which are shown at the same magnification in Fig. 17 and 18. Critical examination shows the sizes in descending order to be bushy stunt, tobacco necrosis, tobacco mosaic A-type preparation. This is in keeping with the fact that their sedimentation constants are 132, 112 and approximately 30×10^{-13}.

In the B preparation (Fig. 4) rods are undoubtedly present, although these are very short, and there is a considerable proportion of the A-type particles. In C-type preparations (Fig. 5) the majority of particles are small rods, some long rods are present, and the proportion of A-type particles is very much reduced. Finally, in D-type preparations long rods predominate, whilst A-type particles are present only in small proportions, although not entirely absent. Indeed, even in preparations containing extremely long particles, such as Fig. 8, it is possible to find a few of what appear to be A-type particles.

Although it is possible to obtain A-type preparations from mincer-extracts which give micrograms identical with Fig. 3, in general the virus prepared from these is in a more highly aggregated state than that obtained from mill extracts, as can be seen by a comparison of Fig. 1 and 2 with Fig. 5 and 6. In these micrograms (Fig. 1 and 2) the C-type contains some particles longer than those shown in the D-type. This is possible for two reasons. In the first place the micrograms were prepared from fractionations made on two separate occasions, the bulk from which Fig. 1 was separated being on the average more aggregated than that from which Fig. 2 was prepared. In the second place, although Fig. 1 contains single particles which are longer than many in Fig. 2, it also contains many more which are shorter, i.e. its average particle length is less. This pair of micrograms illustrates very well the extreme difficulty of preparing homogeneous tobacco mosaic virus, and the danger of assuming that because two preparations have been made from similar starting material, by similar preparative manipulations, they are necessarily identical in so far as particle size, particle size distribution and properties which depend on these are concerned.

Bawden and Pirie (1945) showed that one of the most effective means of causing aggregation was incubation with phosphate and trypsin. Fig. 7 shows the effect of incubating a B-type preparation with phosphate and trypsin (2 g./l of virus, 2 g./l phosphate, pH 6·0, and 0·13 g./l of dialysed B.D.H. trypsin, 2 hours at 37° C.). The average particle length has increased considerably. The ultimate results of incubation with trypsin vary with the nature of the starting material and the exact conditions of incubation. Under favourable conditions the aggregation proceeds to very great particle lengths. Thus Fig. 8 and 9, of trypsin incubated D-type preparations, show particles 100 or more times as long as they are wide. In these preparations it is very difficult to estimate the particle size because of the pronounced tendency to form " mats " of the type of Fig. 9.

The largest single particles that we have observed passed right across the field of view and must have been more than 5μ long.

As Bawden and Pirie have indicated, the smaller fractions are unstable and pass readily into forms with larger particles, the change being accelerated by the presence of salts and other materials present in sap. The extreme ease with which aggregation occurs is illustrated by Fig. 10. This is an almost salt-free B-type preparation, initially giving micrograms indistinguishable from Fig. 4, but which had been kept for 3 weeks in the refrigerator at 4° C. Considerable aggregation has occurred even under these conditions. Subsequent treatment

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DESCRIPTION OF PLATES.

All figures are electron micrograms taken with an R.C.A. (type EMB. 2) instrument. Fig. 1–12 were made with an accelerating potential of 60 kV., and the remainder at 45 kV. Magnifications on the negatives of the former were approximately 13,000 ×, and of the latter about 16,000 ×. Micrograms were enlarged photographically either 3 × or 2½ ×, and have been reduced in reproduction to give a magnification of about 26,000 ×. It should be emphasized that these figures are approximate only. In determining the size of any particular particle, we find it necessary to correct for errors which may amount to as much as ± 20 per cent, and corrections vary with the angular position of the particle. These will be discussed in a subsequent paper.

Tobacco Mosaic Virus.

Preparations shown in Fig. 1–6 were separated by fractionation in the ultracentrifuge and have received no other treatment. Type A sedimented most slowly, D most rapidly, and B and C were intermediate.

FIG. 1.—C-type from mincer extract.

FIG. 2.—D-type from mincer extract.

FIG. 3.—A-type from mill extract.

FIG. 4.—B-type from mill extract.

FIG. 5.—C-type from mill extract.

FIG. 6.—D-type from mill extract.

FIG. 7.—Mill extract C after treatment with trypsin and phosphate (*cf.* Fig. 5).

FIG. 8 and 9.—Mill extract D treated with trypsin and phosphate (two fields from the same preparation).

FIG. 10.—Mill extract B kept 3 weeks at 4° C. (*cf.* Fig. 4).

FIG. 11.—Mill extract B kept 3 weeks at 4° C. and then treated with phosphate (*cf.* Fig. 10).

FIG. 12.—An old and much aggregated preparation evaporated to dryness and re-wetted 3 times.

FIG. 13.—Crude sap. Rods are visible among small particles of normal plant constituent.

FIG. 14.—Crude sap (as Fig. 13) heated to 70° C. for 10 min.

Potato X Virus.

FIG. 15.—Crude sap. Rods are seen amongst the normal plant constituents.

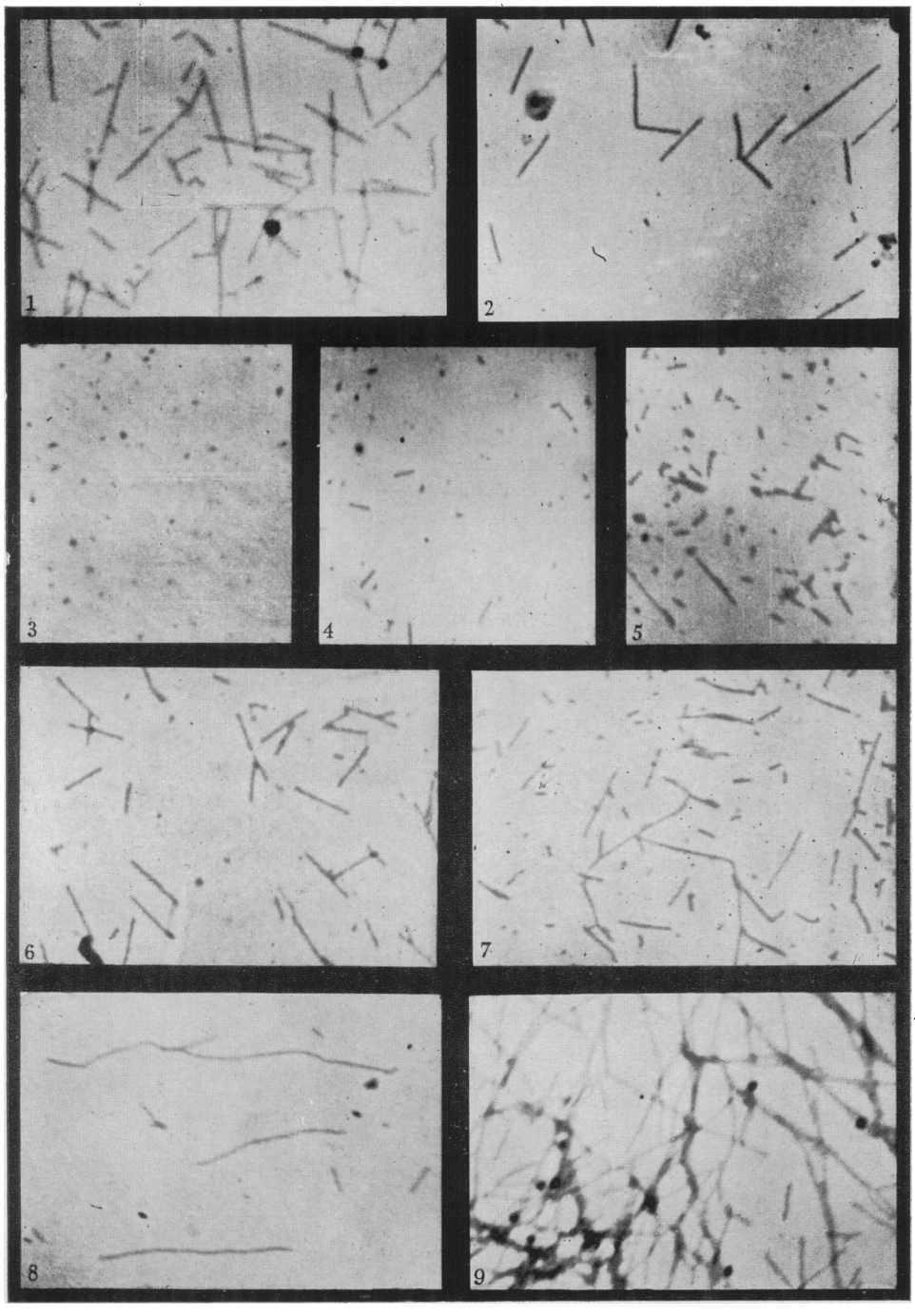
FIG. 16.—Purified preparation.

Tobacco Necrosis Virus.

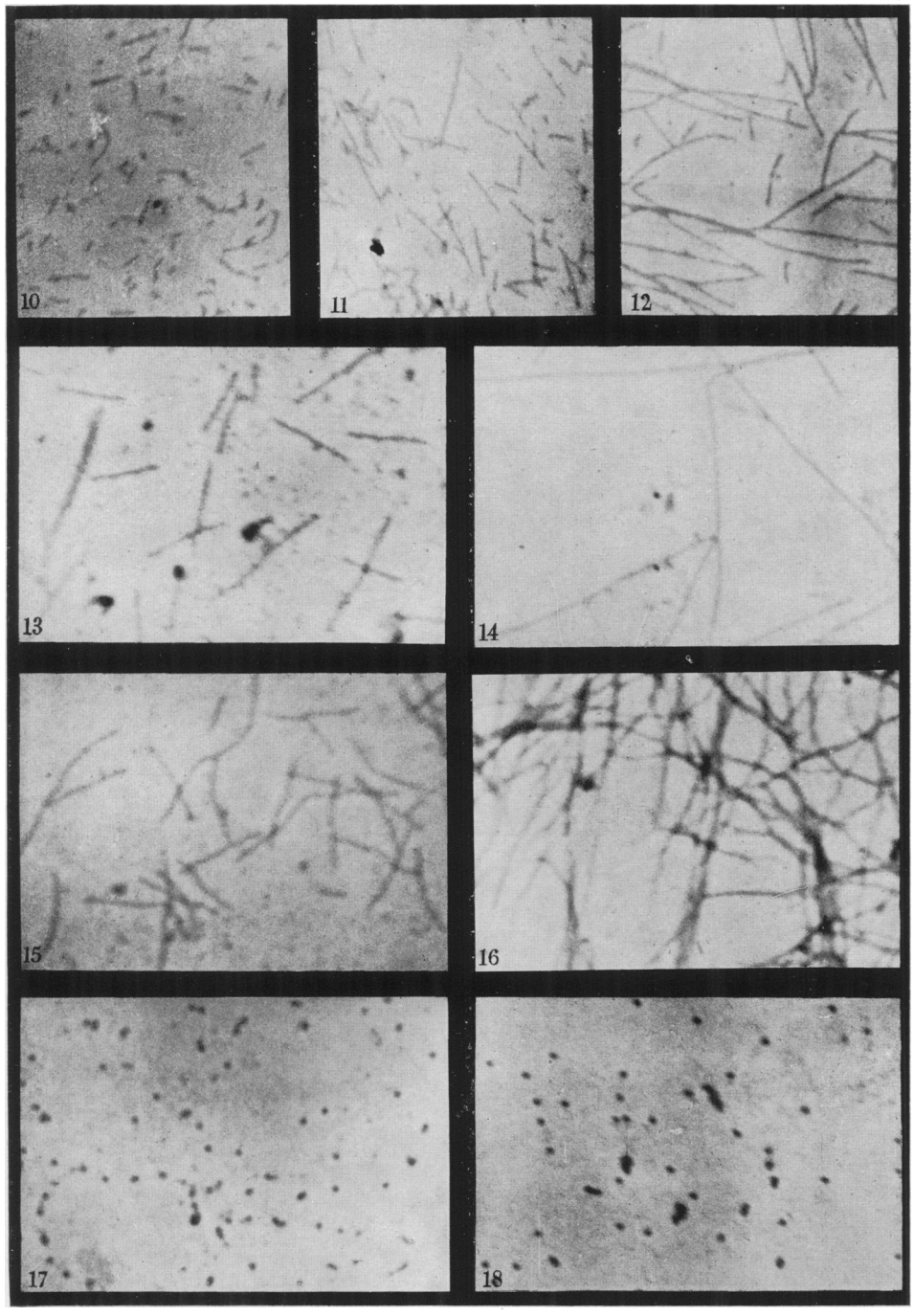
FIG. 17.—A purified preparation of mixed strains.

Tomato Bushy Stunt Virus.

FIG. 18.—Purified preparation.



Crook and Sheffield.



Crook and Sheffield.

with phosphate (2 g./l, pH 6·0, 2 hours at 37° C.) causes aggregation to proceed further to the stage shown in Fig. 11.

One difficulty of interpreting electron micrograms arises from the lack of precise knowledge of the effect of drying on the virus particles. Bawden and Pirie (1937) have shown that drying inactivates the virus and reduces the anisotropy of flow. To try and arrive at some estimate of the effect of drying, a mixed batch of old trypsin-treated preparations was allowed to evaporate on the laboratory bench, the dried material taken up in water and the process repeated twice more. Thus, including the drying incidental to the making of the microscope specimen, this material had been dried four times in all compared with once for a normal preparation. Fig. 12 shows that it has suffered no serious disaggregation. Many small particles are present, but they are not sufficiently increased in number, nor the larger ones sufficiently diminished for there to be any confusion with, say, an A-type preparation.

Aggregation of a similar type occurs not only in purified preparations, but in crude sap. Indeed, conditions in sap are such as to favour aggregation, and treatments applied in this medium lead to a large degree of aggregation. For example, Fig. 13 shows particles of tobacco mosaic virus as they appear in crude sap. Small particles may be difficult to see because of the background of normal leaf material, but the larger particles are sufficiently distinctive to be easily identified. The effect of heating this preparation to 70° C. for 10 mins. is shown in Fig. 14. Here the rods have elongated so greatly that they extend across the whole field.

That this linear aggregation of particles is not confined to tobacco mosaic virus but occurs in at least one other virus with rod-shaped particles is illustrated in Fig. 15 and 16. The former is a microgram of potato virus X as it occurs in crude tobacco sap. The latter shows the state of increased aggregation which may result when the virus is purified by certain methods.

Fig. 17 and 18, tobacco necrosis and tomato bushy stunt viruses, are included, mainly for comparison with A-type preparations of tobacco mosaic virus. However, they do show the character of these virus particles very well, possibly better than other micrograms so far published. Whether the slight tendency to asphericity noticeable in these micrograms, particularly in that of tobacco necrosis, is real or whether it is due to clumping of the particles, distortion during drying, or some defect in the microscope optical system cannot as yet be ascertained.

DISCUSSION.

The results described here show that there is no basis for assigning a " length " to " the molecule " of tobacco mosaic virus from electron microscope measurements as has been attempted by several workers. Even in a single preparation, the length of the individual particles varies considerably, and by varying the conditions under which the preparation is made the average size of the particles can be varied from those shown in Fig. 3 to the " mat " of Fig. 9. The present data also show that Markham, Smith and Lea (1942) were in error when they concluded that " an estimate of size can be given with some confidence," although the data available at that time were in some measure self-consistent.

Stanley and Anderson (1941) also had variable length particles to deal with.

Thus, their data were obtained by measuring "all of the 58 particles in two selected fields." The selection would naturally be to obtain as many likely looking particles as possible in a field, i.e. to select uniform fields. Yet even under these conditions, on a weight basis only 50 per cent of the material occurred as particles of 280 mμ., and their histogram shows that fewer than half the particles were of this length. Frampton (1942) has pointed out that there are particles as short as 37 mμ. in their micrograms, and suggested that these might be the basic units from which the larger rods were built up. However, the A-type preparations shown here obviously have particles smaller than this—many being at least as short as the diameter of the rods (15 mμ.). This is in agreement with Rawlins' (1942) criticisms of Frampton when he pointed out that the regularities in Frampton's measurements were not sufficient for any conclusion to be drawn about the size of a basic unit. However, there was no basis for Rawlins' argument that because of these irregularities in length he was dealing, not with a "molecule," but an "organism." If this argument were followed to its conclusion we should be endowing with "life" the particles of many a thermoplastic polymer merely because they were not uniform in length.

Stanley and Anderson, in discussing the small particles which appear in their micrograms, concluded that their true nature and significance was unknown, but implied that they might have been produced at the time the specimen was mounted, since there was no evidence for their presence from ultracentrifugal analysis. Rawlins *et al.* (1946) made a similar suggestion. From the data presented here it can be seen that although drying may cause a small amount of disaggregation (Fig. 12), many preparations contain exclusively short particles. We therefore conclude that short particles occurring in long particle preparations are not merely artefacts or breakdown products, but normally occurring constituents. The relationship between these particles and the longer rods has been fully discussed by Bawden and Pirie (1945).

It is unlikely that the differences between previous results and those reported here are due to any basic differences between the virus strains. Stanley and Anderson (1941) concluded from the difference between their measurements and those of Melchers, Schramm, Trurnit and Friedrich-Freska (1940) that different virus strains have different particle lengths. That postulate appears to be unnecessary, since the particle length of a preparation from a single strain depends so closely upon its previous history that there is adequate scope for the observed variations within it. Now that this variation has been disclosed, a large accumulation of data will be necessary before any variation between strains can be adequately demonstrated.

As all the micrograms of tobacco mosaic virus so far published show considerable variation in the size of the particles, it is curious that this variation has been largely ignored. Such neglect is probably due partly to the preconceived idea that viruses are macromolecules and therefore should be uniform, and partly because most workers have not examined a sufficient number of micrograms to emphasize the variability. Thus Stanley and Anderson (1941) base their findings on examination of two selected fields from a single preparation, and Lauffer (1944) on one micrograph from each of two preparations. Before such measurements are valid two conditions must be fulfilled : the field photographed must be representative of the specimen, and the specimen itself representative of the preparation as a whole.

The former of these conditions is fulfilled by making a number of records of each specimen. However, the latter condition is much more difficult to fulfil satisfactorily because of several phenomena connected with the drying of virus solutions. Dilutions of the order of 1 in 10^5 or 10^6 are necessary to obtain particles sufficiently dispersed to be able to distinguish individuals. At these dilutions, a considerable fraction of the virus is in the form of a surface film which is likely to contain a different ratio of large to small particles than that in the bulk phase. If, by chance, portions of this surface film are examined, a spurious ratio of small to large particles may be recorded. Again, even at dilutions of 1 in 10^6, virus tends to move to the outer fringe of the drop during drying, forming a " mat " too dense for the electron beam to penetrate, while the area occupied by the centre of the drop is left almost barren of particles. It is not known whether this movement is due to the surface film or whether it affects small and large particles equally, and in any event, it often makes difficult the preparation of a specimen with concentrations of virus at the centre of the field optimum for viewing. In the present work we have attempted to overcome these disabilities by using both centrally placed drops and drops placed asymetrically so that the region of " mat " formation crosses the field of view. We have thus been able to examine both regions. While we do not claim that this is an adequate solution of the problem, it does ensure that the virus is examined under a fairly wide range of conditions. The use of a sufficient number of preparations and an adequate number of photographs from each improves the chances of obtaining representative micrograms. The present material has been selected as being the most representative set from nearly 700 negatives made from 29 different preparations of tobacco mosaic virus.

In certain of the preparations (Fig. 7, 8) apparently continuous rods may not be straight, but may change their direction abruptly several times. It seems probable that these discontinuities are the points at which shorter particles have joined, and may correspond to those shown in the excellent shadow-cast micrograms of Williams, Robley and Wyckoff (1945). Occasionally an actual break in the rod is visible (Fig. 6).

The data presented here include no evidence regarding a possible basic unit from which the particles are built nor of the size of such a unit. From an examination of micrograms of A-type preparations, however, it can be concluded that such a unit, if it exists, is small, possibly too small even to be seen or measured in the electron microscope. This follows from the fact that such preparations contain material of length at least as short as the diameter of the rods. Nor is evidence given here as to whether the aggregation proceeds continuously or discontinuously, i.e. whether or not there are aggregates of certain lengths which are more stable than others. It seems quite likely that this is so, and indeed it would explain the frequency with which a length of approximately 300 mμ. has occurred in measurements of tobacco mosaic virus. It is hoped shortly to present evidence on both these points, together with some numerical data on the effect of preparative history upon particle length.

In conclusion, it is worth emphasizing that since we have demonstrated linear aggregation in at least one other virus with rod-shaped particles, namely, potato X virus, all figures for particle size of other rod-shaped viruses (e.g. poliomyelitis) must remain suspect until it is proved that they are not being invalidated by the same phenomenon.

SUMMARY.

Preparations of purified tobacco mosaic virus separated according to Bawden and Pirie's (1945) method of differential ultracentrifugation have been examined in the electron microscope. Micrograms of the most slowly sedimenting fraction (A) contain particles most of which are no longer than their diameter (15 mμ.), and these particles are compared with the spherical viruses, tobacco necrosis and tomato bushy stunt. As sedimentation rate increases (types B–D) the specimens contain rods of increasing length. Micrograms of crude sap show rods mixed with other plant constituents. Keeping the virus for 3 weeks at 4° C. causes some particles to aggregate, and aggregation proceeds further during treatment with phosphate. Heating under certain conditions to 60° C. or more or incubating at 37° C. with trypsin produces many rods longer than 5 μ. Drying causes no serious disaggregation.

Particle length varies also in potato X virus.

In no preparation of a rod-shaped virus were the particles all of uniform length, and in tobacco mosaic virus some particles of the A-type were always present. All evidence indicates that average rod length is dependent on preparative treatment.

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