ELECTRON MICROSCOPE STUDIES ON PLANT VIRUSES I

By

TEIKICHI FUKUSHI and EISHIRO SHIKATA

Introduction

It is well known that the limits of microscopic visibility have been exceedingly extended by the invention of an electron microscope with high resolving powers, offering the possibility of securing micrographs of viruses and to establish their shape and size with some precision. KAUSCHE et al. (1939) were the first to demonstrate the viruses of tobacco mosaic and potato latent mosaic by the electron microscope. They showed that the particles of tobacco mosaic virus are rod-shaped, 150–300 mμ long and 15 mμ wide. After that a considerable number of publications have accumulated, dealing with the morphological aspects of plant viruses. Since detailed accounts of the electron microscopy of plant viruses have been given in recent works of RUSKA (1950), BAWDEN (1950), SMITH (1951) and the senior writer (1952), a superfluous repetition here can be avoided. Nevertheless a brief account will be given in connection with recent advances in Japan in the electron microscope studies on plant viruses.

It is worthy of note that SUGATA in the Faculty of Engineering, Osaka University succeeded in 1939 to construct an electron microscope of magnetic field type and 4 years later he revealed by means of this electron microscope, rod-shaped particles in the suspension of tobacco mosaic virus, although the micrographs he obtained have not been published. The metallic shadow-casting introduced by WILLIAMS and WYCKOFF (1944, '45, '46) provides a method for increasing contrast in electron microscopic preparations. Applying

This research was supported in part by a Grant for Scientific Research from the Ministry of Education.

The writers are indebted to Messrs. H. TSUCHIKURA, H. YOTSUMOTO and S. TAKAHASHI for their assistance in making electron micrographs of viruses.

this technique HIGASHI (1949) secured micrographs of tobacco mosaic virus and FUKUSHI et al. (1951, '52) those of tobacco mosaic virus and potato virus X. Measurements of centrifugally purified tobacco mosaic virus have been given by HIDAKA and MURANO (1952) while FUKUSHI and SHIKATA (1952) published information on the size and shapes of tobacco mosaic virus and potato virus X which had been purified by chemical means. HIDAKA and KIRIYAMA (1952, '53) have reported on the morphological change, pathogenicity and serological activity of tobacco mosaic virus treated with heat, ultrasonic waves, formaldehyde, the extract form the leaves of *Phytolacca americana* L. and "bukulin,, extracted from the sclerotia of *Pachyma cocos* FRIES. FUKUSHI and SHIKATA (1953) also informed briefly on the transformation of tobacco mosaic virus induced by heat. In the present paper a more detailed account will be given on the results of the writers' electron microscope studies on tobacco mosaic virus and potato virus X.

Materials and Methods

Purification of tobacco mosaic virus. Five or six-leaved young plants of *Nicotiana tabacum* var. White Burley or *N. sylvestris* were inoculated with tobacco mosaic virus. About 2–3 weeks later affected leaves were harvested and frozen at −20°C. They were ground in a mortar while frozen and after thawing the sap was expressed through two thicknesses of gauze. The virus was isolated and purified by means of chemical fractionation in the following way. The expressed sap was centrifuged at 3,000 r. p. m. for 30 minutes and the supernatant fluid was then one-quarter saturated with ammonium sulfate. After centrifugation the precipitate was suspended in water and centrifuged again to remove insoluble materials. This procedure was repeated 2–4 times and the fluid containing the virus was then brought to pH 3.3 by the addition of dil. ClH. The resulting precipitate was dissolved in a volume of water or phosphate buffer of pH 7.0, equal to one-tenth of the original sap and centrifuged at 10,000 r. p. m. for 30 minutes. The supernatant fluid of the latter, that is the virus suspension in phosphate buffer of pH 7.0, provided material suitable for electron microscopic examination. Further purification of the virus, however, was attempted by tryptic digestion and electrophoretic fractiona-

tion. The virus suspension was incubated with 0.2% trypsin at pH 8 and 37°C. After 24 hours' digestion the virus in the fluid was precipitated repeatedly by ammonium sulfate and by the addition of dilute acid. The resulting virus suspension was purified by electrophoretic fractionation employing the Todd U tube electrophoresis cell (27). Buffer solutions used were mixtures of 0.2 M disodium phosphate and 0.1 M citric acid, the pH values of which were approximately 4, 5, 6, 7 and 8. A direct current circuit giving 100 volts was used; the current varied from 8 to 10 milliamperes; the duration of electrophoresis was 4 hours at each pH. After electrophoresis the liquid from the anode was taken into a cellophane sac and dialyzed overnight against tap water. The final preparation was entirely colorless; it was stored at 1–2°C.

Heat treatment. Heat treatment of the virus was performed in two different ways. In one series of experiments the virus suspension in a test tube was immersed in a water bath held at the desired temperature. Immediately after the heat treatment, the tubes were put into the water. In another series of experiments the virus suspension was heated on the collodion films on electron microscopic specimen screens which were kept in saturated steam at the desired temperature. In this case a water bath 15 cm. in diameter containing water to half its depth was tightly covered with a glass lid. A tripod was placed in the water, the top being 2–3 cm. above the water surface. A thermometer was inserted in the hole at the top of glass cover and used to measure the temperature of the steam around the specimen screens on the tripod. One platinum-loopful of virus suspension was put onto the collodion film on a specimen screen and promptly placed on the tripod in the steam at the desired temperature. Only 30 to 40 seconds were necessary to regain the desired temperature of the steam in the vessel. After the heat treatment the screen was cooled in water and thereafter dried at room temperature.

Purification of potato virus X. This virus was isolated from affected tomato and tobacco plants and purified by BAWDEN and PIRIE's method reported in 1938. The sap obtained from the tomato plants could be readily purified by precipitation with ammonium sulfate while for the juice from the tobacco plants which contained impurities in large quantities further purification by precipitating the virus at pH 4.5 gave suitable preparation.

Preparation for electron microscopic examination. Purified suspension of the virus, either heat treated or untreated, was placed on collodion films on specimen screens, dried and shadowed with chromium; the preparation was examined in HU-Type 4 or 7, or JEM-Type 4 electron microscope. When virus suspension was heated on the collodion film on a specimen screen as above stated, the screen was cooled in the water, dried and shadowed.

Experimental Results

I. Shape and size of potato virus X

Electron micrographs of potato virus X, mottle strain revealed that the virus particles were sinuous rods of filaments as shown in Pl. I, Figs. 1 and 2. Fifty-nine per cent and 74 per cent of the virus particles obtained from tomato and tobacco plants, respectively, were 400–500 mμ long and about 10 mμ wide. Most of the virus particles were from 425 to 450 mμ in length as shown in Diagram 1.

KAUSCHE et al. (1939) were the first to demonstrate this virus by the electron microscope. Subsequently TAKAHISHI and RAWLINS (1946) and WYCKOFF (1949) described and illustrated the elementary filament of this virus. According to TAKAHASHI and RAWLINS virus particles of ring spot and mottle strains of potato virus X are indistinguishable in form and size, ranging from 200 to 650 mμ in length and 14 to 18 mμ in width, but a high proportion of the particles have length between 500 and 600 mμ and a mean width of 16 mμ. WYCKOFF also states that virus filaments in a purified preparation have been found between 500 and 600 mμ long although there are others that are far longer. BODE and KÖHLER (1952) wrote that the great majority of potato virus X particles were of uniform length, those of strain X^N and X^E being 600 to 620 and 560 to 580 mμ, respectively.

LORING (1938) obtained 43.9 : 1 for the ratio of particle length to width, calculated from viscosity measurements. He estimated the molecular weight to be 26×10^6 based upon specific volume of 0.73 and a sedimentation constant of 113, suggesting that a cylindrical particle of above mentioned molecular weight, specific volume and a ratio of length to width would have a diameter of about 9.8 mμ and a length of about 433 mμ. These values are in

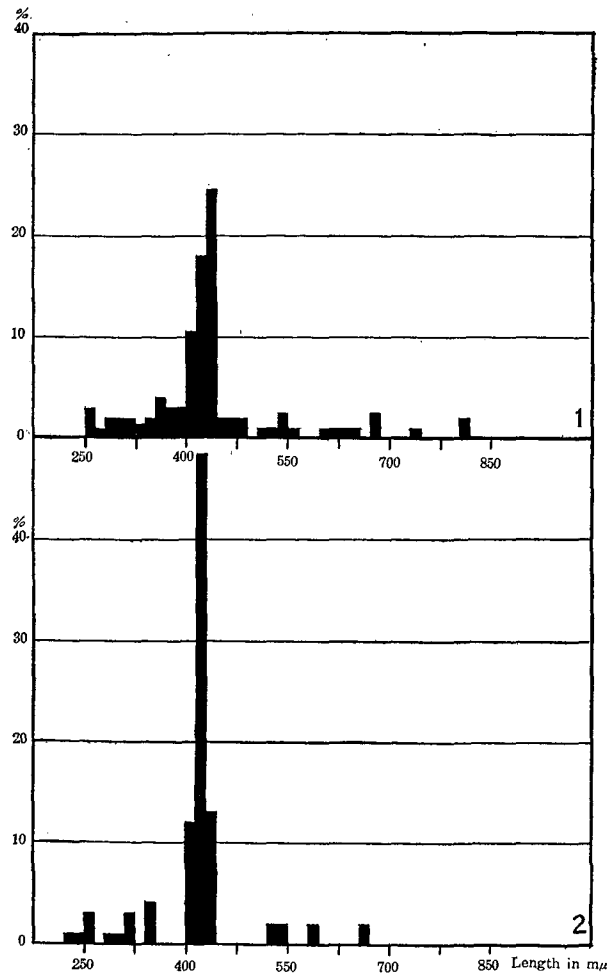


Diagram 1. Size distribution of virus filaments in the suspension of chemically purified potato virus X. 1: Virus filaments isolated from an affected tomato plant. 2: Virus filaments from a diseased tobacco plant.

good agreement with those obtained from electron micrographs in the present work, as above stated.

II. Morphological change of tobacco mosaic virus caused by heat

Series I.

The virus suspension purified by repeated salting out with

ammonium sulfate and precipitation with acid was diluted with distilled water at concentrations of 1 in 10 or 100 and dialyzed overnight against tap water to remove salts. The final liquids were distributed into test tubes and heated at 70°, 80°, and 90°C for 10 minutes. Upon examination of these preparations by the electron microscope it was found that the virus particles aggregated so densely that the shape of virus particle was not distinctly recognizable. Particularly in the preparations heated at temperatures higher than 80°C. coagulated amorphous deposits were abundantly seen intermingled with the virus rods. Further purification of the virus, therefore, was carried out by tryptic digestion and electrophoretic fractionation as mentioned before. Such preparations, however, also gave similar results. It was conceivable that virus particles and impurities of proteinous nature may be coagulated into masses by heat and precipitated altogether. Accordingly the virus suspension was heated on the collodion films on specimen screens which were kept in saturated steam at the desired temperature. A small amount of virus suspension, containing approximately 0.02% virus, which had been dialyzed against tap water to remove electrolytes was heated on the collodion film at 60°, 70°, 80°, 90° and 95°C for 10 minutes, respectively. About 3.5 ml of the same preparation were placed in a glass tube about 8 cm long and 0.5 cm in diameter, stoppered at both ends and heated in steam at the desired temperature for 10 miuntes. After cooling the infectivity of the suspension was tested on the leaves of *Nicotiana glutinosa* plants.

As shown in Pl. II, Figs. 2–6 the virus particles heated at 70°C for 5–40 minutes did not disperse uniformly but showed a tendency to aggregate into networks, those heated at 80° for 5-30 minutes (Pl. III, Figs. 7–11) produced abundant globular deposits among aggregated virus particles; the specimens heated at 90° or 95° for 10 minutes (Pl. III, Fig. 12 and Pl. IV, Fig. 13) showed coagulated amorphous masses and small globular deposits scattered in the field, no rod-shaped virus particles being found. On the other hand the infectivity of virus suspension heated at 80° for 10 minutes remarkably decreased while heating at 90° for 10 minutes rendered the virus suspension entirely noninfective as shown in Table 1. Generally speaking, the virus particles in the suspension which had been dialyzed to remove electrolytes showed the tendency

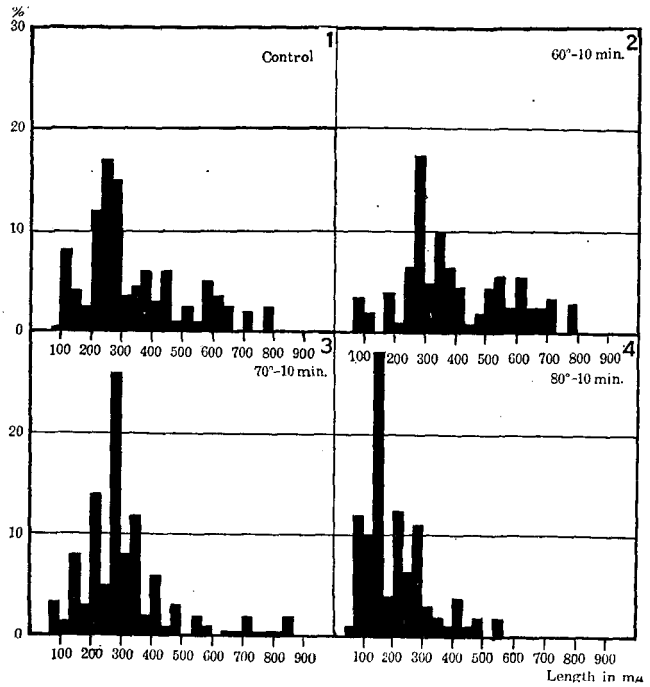


Diagram 2. Size distribution of virus rods in the suspension of chemically purified tobacco mosaic virus (TMV) heated at higher temperatures. (Dialyzed virus suspension) 1: Unheated control. 2: TMV heated at 60°C for 10 min. 3: TMV heated at 70° for 10 min. 4: TMV heated at 80° for 10 min.

toward end to end aggregation, being longer than 280 mμ. When such a preparation was heated, the virus particles were apt to aggregate into networks rendering difficult the measurement of the length of individual particles, even when diluted to a considerable extent. Measurements of the length of virus particles are shown in Diagram 2 which indicates that particles around 140 mμ suddenly increase and those longer than 280 mμ decrease in the suspension heated at 80° for 10 minutes.

In another series of tests the virus preparation was heated at 70° and 80° for 5, 10, 20, and 30 minutes (v. Table 1, Pl. II, Figs. 2–6 and Pl. III, Figs. 7–11, and Diagram 3). There was a slight decline in the infectivity of the virus in the suspensions which were heated at 70° for 30 and 40 minutes; furthermore was found in such suspensions that virus particles shorter than 100 mμ increased

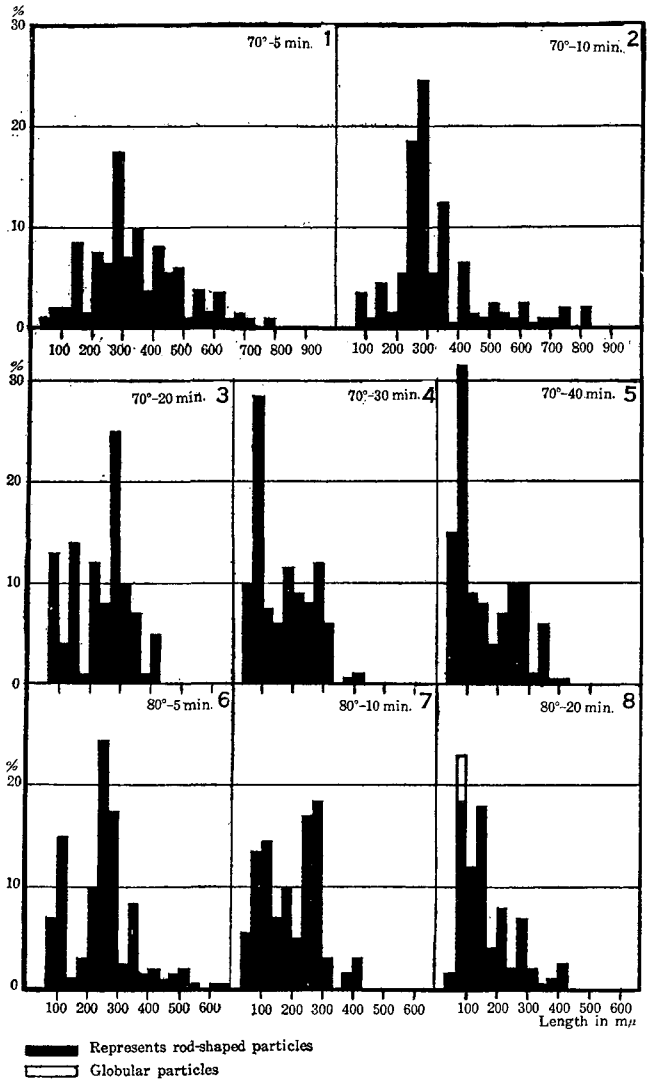


Diagram 3. Size distribution of virus particles in the suspension of chemically purified tobacco mosaic virus (TMV) heated at higher temperatures. (Dialyzed virus suspension) 1: TMV heated at 70° for 5 min. 2: TMV heated at 70° for 10 min. 3: TMV heated at 70° for 20 min. 4: TMV heated at 70° for 30 min. 5: TMV heated at 70° for 40 min. 6: TMV heated at 80° for 5 min. 7: TMV heated at 80° for 10 min. 8: TMV heated at 80° for 20 min.

while those equal to or longer than 280 mμ were reduced in number when viewed in the electron microscope. Heating of the virus suspension at 80° for 5 minutes apparently caused no effect upon the infectivity of the virus but resulted in the aggregation of the latter into networks, while 10 minutes' heating caused a decrease in virus infectivity and corresponding increase of small globular deposits and virus particles shorter than 280 mμ in length. The virus suspension heated at 80° for 20 minutes retained only a slight infectivity. Electron micrographs of such a suspension revealed small globular fragments about 100 mμ in diameter scattered in the field, although a few virus rods, some of which were apparently divided into small segments, were found still remaining in another field. When the virus suspension was heated at 80° for 10 minutes and centrifuged at 3,000 r.p.m. for 30 minutes, the virus rods were shown to be very few in number in the supernatant fluid suggesting that most of virus particles in the suspension might have been coagulated by heat and eventually precipitated.

Series II.

As suggested by TAKAHASHI and RAWLINS (1948) and TAKAHASHI (1949), tobacco mosaic virus particles show linear aggregation less frequently in the buffer solution of pH 7.0. In this series of experiments, therefore, phosphate buffer of pH 7.0 was used instead of water in the final process of purification and concentration of the virus. Purified suspension of the virus, however, was diluted with distilled water to 1 : 10, because otherwise crystals of salts were separated in the suspension when dried on collodion films and the observation of virus particles by the electron microscope was hindered. The virus particles in such preparation are scattered uniformly and are not coagulated into a network as a result of heating. Accordingly heating was performed by immersing a test tube which contained the virus suspension into the water bath at a desired temperature. Subsequently the test tube was cooled in the water and a small portion of the virus suspension was used to provide the preparation for the electron microscope, while the other portion was used to inoculate leaves of *Nicotiana glutinosa* plants for the assay of infectivity. Such a suspension, unlike the dialyzed virus suspension, did not coagulate when heated and produced no amorphous masses of deposit which might have been an

obstacle to the electron microscope observation. Further purification by tryptic digestion and electrophoretic fractionation was not indispensable. Subsequent experiments, therefore, were carried out with the virus purified by salting out and precipitation at the isoelectric point, dissolved in buffer mixture of pH 7.0 and finally centrifuged at 10,000 r.p.m. for 30 minutes. The dialyzed virus suspension diluted 1 : 10–100 remained clear even when heated at 90° whereas the suspension of pH7.0 at the same virus concentration began to become slightly turbid when heated at 70° and became more or less cloudy at 75° or 80°C. If a small amount of physiological saline was added to the dialyzed virus suspension the latter as well as the concentrated virus suspension became turbid when heated.

The infectivity of virus suspension began to diminish when heated at 75° for 10 minutes and was entirely lost at 80° for 10 minutes as shown by the results of assay of infectivity, listed in Table 1. As shown in electron micrographs, Pl. IV–V, Figs. 15–24 and Digram 4, the higher the temperatures are the greater the

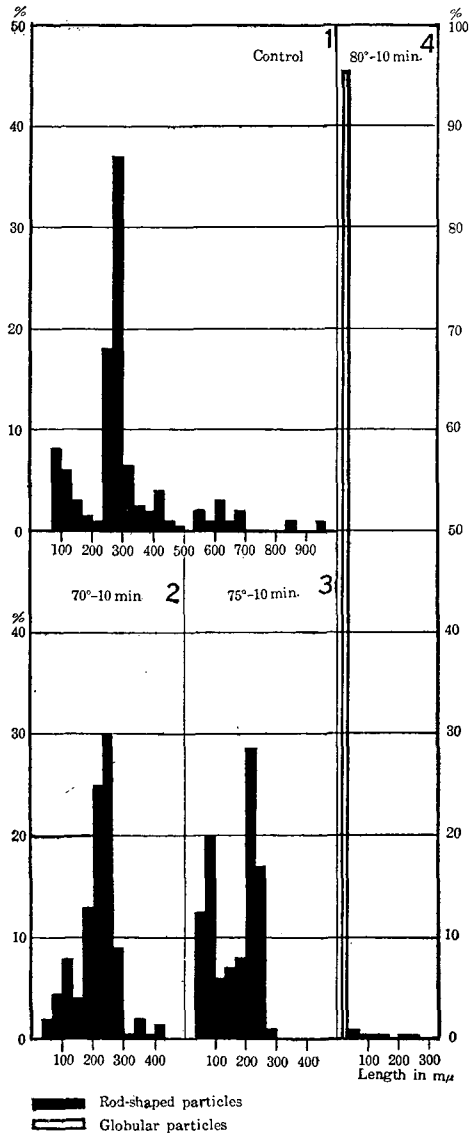


Diagram 4. Size distribution of virus particles in the suspension of chemically purified TMV heated at higher temperatures. (Virus particles dispersed in phosphate buffer of pH 7.0) 1 : Unheated control. 2 : TMV heated at 70° for 10 min. 3 : TMV heated at 75° for 10 min. 4 : TMV heated at 80° for 10 min.

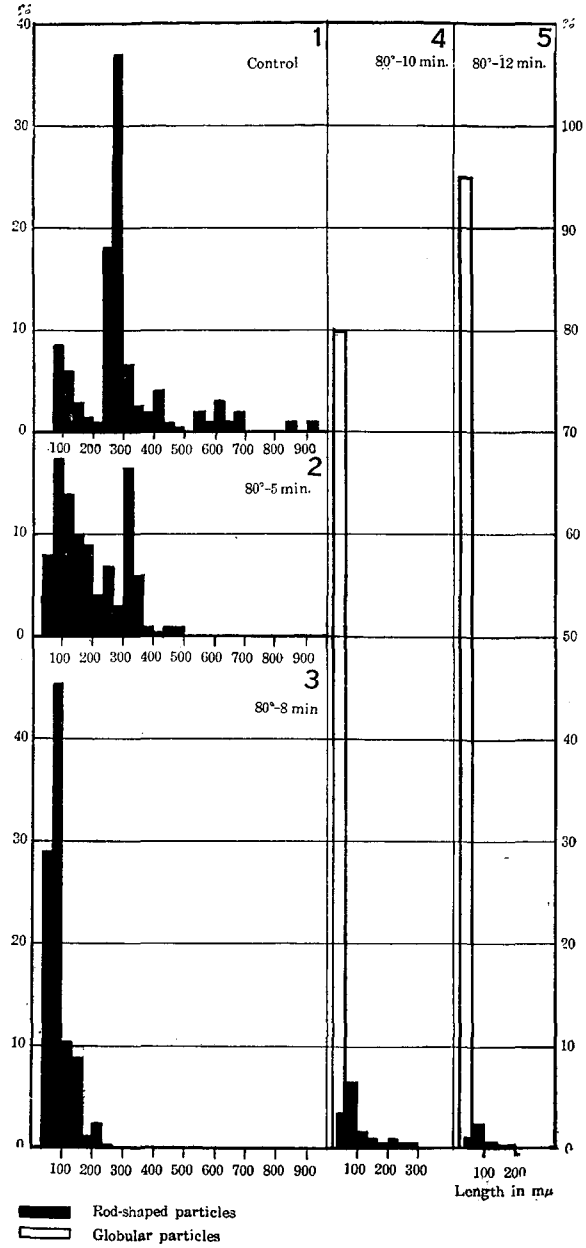


Diagram 5. Size distribution of virus particles in the suspension of chemically purified TMV heated at higher temperatures. (Virus particles dispersed in phosphate buffer of pH 7.0) 1: Unheated control. 2: TMV heated at 80° for 5 min. 3: TMV heated at 80° for 8 min. 4: TMV heated at 80° for 10 min. 5: TMV heated at 80° for 12 min.

number of comparatively shorter virus particles in the heated suspension. When heated at 80° the virus suspension revealed numerous globular particles 20–50 mμ in diameter and a few virus rods some of which were apparently divided into small segments. The smallest rods were approximately 20 mμ in length. In the virus suspension heated at 85° no virus rods were found and some of the globular particles aggregated into small masses.

As it became evident that the virus particles might be transformed at the temperatures around 80°C, the virus suspensions were heated at 80° for 5, 8, 10, 12 and 15 minutes to investigate more minutely the change in the form of virus. The electron micrographs obtained, Pl. V, Figs. 19–24 demonstrate that prolonged heat treatment of the virus suspension is accompanied by an increase in the number of shorter rods which are eventually replaced by globular particles 20–50 mμ in diameter. The shortest rods are about 20 mμ in length and the particles 40–50 mμ long are found more abundantly (v. Digram 5). It is difficlut to discern accurately whether the particles shorter than 20 mμ are spherical or rod-shaped, particularly in shadowed preparations. In the virus suspension heated at 80° for 20 or 30 minutes it appears that the above-mentioned globular particles aggregate into closely packed clusters.

In another series of tests the virus particles suspended in the buffer mixture of pH 5.2 were exposed to heat at 75°, 80°, 85° and 90°, respectively for 10 minutes. In these cases also the virus particles were found not to coagulate into a network but to disperse uniformly in the suspension. When virus suspension was heated at 80° the number of comparatively shorter rods increased in it while in the suspension heated at 85° globular particles and shorter rods of 50–100 mμ in length were produced abundantly (v. Diagram 6).

It is evident from these results that the change of shape of tobacco mosaic virus particles caused by heat depends upon the state of the virus suspension. In the suspension which had been dialyzed against water to remove the salts involved the virus particles showed a tendency towards aggregation when heated at 60° or 70° and with rising temperature there was an increase in the number of shorter rods or broken virus particles which at length turned into small particles of globular appearance. On the other hand, the virus particles dispersed in buffer mixture of pH

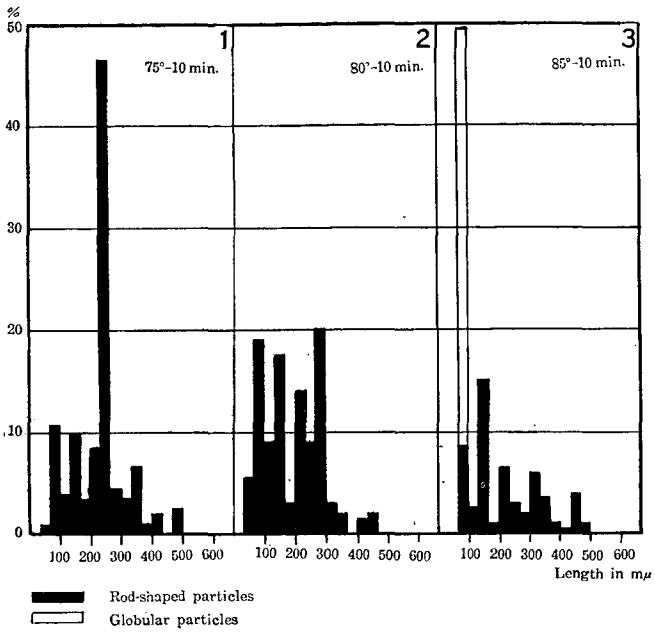


Diagram 6. Size distribution of virus particles in the suspension of chemically purified TMV heated at higher temperatures. (Virus particles dispersed in phosphate buffer of pH 5.2) 1: TMV heated at 75° for 10 min. 2: TMV heated at 80° for 10 min. 3: TMV heated at 85° for 10 min.

7.0 or 5.2 did not readily aggregate when heated but even in such a case the suspension exposed to higher temperature revealed an increase in number of shorter particles which would be replaced subsequently by globoular particles.

Infectivity assay and electron microscopic examination of the virus suspension exposed to heat demonstrated that both infectivity and number of comparatively longer virus particles were reduced with the rising of temperature and prolongation of exposure time as shown in the following table. The virus particles suspended in buffer mixture of pH 7.0 were completely inactivated when heated at 75° for 10 minutes whereas those in that of pH 5.2 or in the dialyzed suspension appeared to be more tolerant to heat having retained slight infectivity even after exposure to 80° for 10 minutes.

It is generally accepted that rods of varying length both shorter and longer than 280 mμ are found in the freshly expressed juice of mosaic-diseased tobacco plants as well as in the purified sus-

pension of the causal virus when viewed in the electron microscope (RAWLINS et al. 1946, TAKAHASHI and RAWLINS 1948, '49, SIGURGEIRSSON and STANLEY 1947, OSTER 1947). Several workers believe the infective unit of tobacco mosaic virus to be a rod-shaped particle of around 280×15 mμ, particles shorter than this being presumed to possess little or no virus activity (SIGURGEIRSSON and STANLEY 1947, TAKAHASHI and RAWLINS 1949). There is no satisfactory explanation of the origin of rods shorter than 280 mμ but it is conceivable that they may arise from the breakage of the longer rods because of thermal or mechanical stresses as suggested by SIGURGEIRSSON and STANLEY. KAUSCHE et al. (1941), OSTER (1947) and HIDAKA and KIRIYAMA (1953) state that the rod-shaped particles of tobacco mosaic virus are broken by ultrasonic treatment to form particles of shorter lengths. It is considered of significance that the results of present work have demonstrated the increase of the proportion of shorter virus particles, in the virus suspension exposed to comparatively higher temperatures. As shown in Table 1, about 55% and 85% of the particles were shorter than 230 mμ in the preparation which was subject to 80°C for 10 and 20 minutes, respectively, whereas about 30% and 10% of the virus particles were shorter than 230 mμ in the dialyzed suspension, whether unheated, or heated at 60° for 10 minutes. It appears that a correlation exists between the size distributions and infectivity of virus particles as determined by inoculation on leaves of *Nicotiana glutinosa* plants. As for virus particles in buffer mixture of pH 7.0, nearly 100 % of the particles were less than 230 mμ in length after the suspension had been heated at 80° or 85°C for 10 minutes whereas about 55% and 80% of the particles were shorter than 230 mμ when exposed to 70° and 75°, respectively for 10 minutes. It is obvious from the table that the virus particles heated at 80° for 10 minutes were rendered entirely noninfecticus.

There is no reason to consider that the only effect of heat is to break the virus rods or that heat exerts similar action upon the virus to that induced by centrifugal force and ultrasonic vibrations. Irradiation with ultraviolet light and X-rays is capable of destroying the infectivity of the tobacco mosaic virus without affecting the particle length or serological properties as will be reported in subsequent publications. From the results of the present work, however, it is conceivable that the effect of com-

TABLE 1 Infectivity and particle size of TMV exposed to high temperatures

Temperature and time of exposure	*No. of necrotic spots on *N. glutinosa*	Length of virus particles						
		Shorter than 130 mμ (globular) %	Shorter than 130 mμ (rod-shaped) %	130–230mμ %	230–330mμ %	330–430mμ %	430–530mμ %	Longer than 530 mμ %
Dialyzed virus suspension								
Control (unheated)	185	—	9.5	20.0	35.0	13.5	10.0	12.0
60° — 10 min.	219	—	5.5	5.0	29.0	21.0	7.5	32.0
70° — 10 min.	133	—	5.0	25.0	39.0	20.0	4.0	7.0
80° — 10 min.	54	—	23.0	44.5	20.5	7.0	3.0	2.0
90° — 10 min.	0	—	—	—	—	—	—	—
95° — 10 min.	0	—	—	—	—	—	—	—
Control (unheated)	249	—	—	—	—	—	—	—
70° — 5 min.	228	—	5.0	17.5	31.5	21.5	12.5	12.0
70° — 10 min.	209	—	4.5	11.5	49.0	19.5	6.0	9.5
70° — 20 min.	216	—	17.5	27.0	42.5	13.0	0	0
70° — 30 min.	168	—	46.0	26.5	26.0	1.5	0	0
70° — 40 min.	174	—	54.5	18.0	20.5	7.0	0	0
Control (unheated)	210	—	—	—	—	—	—	—
80° — 5 min.	189	—	23.0	15.0	45.5	10.5	4.5	1.5
80° — 10 min.	69	—	33.5	22.5	39.5	4.5	0	0
80° — 20 min.	6	23.5	32.0	30.0	11.5	3.0	0	0

Virus in buffer mixture of pH 7.0								
Control (unheated)	247	—	12.0	6.5	62.0	8.5	3.0	8.0
70° — 10 min.	161	—	14.5	42.0	39.5	4.0	0	0
75° — 10 min.	38	—	38.5	43.5	18.0	0	0	0
80° — 10 min.	0	95.0	2.0	2.0	1.0	0	0	0
85° — 10 min.	0	100.0	0	0	0	0	0	0
Control (unheated)	416	—	—	—	—	—	—	—
80° — 5 min.	213	—	40.5	23.0	26.0	8.5	2.0	0
80° — 8 min.	86	—	82.5	16.0	1.5	0	0	0
80° — 10 min.	0	80.0	15.5	3.5	1.0	0	0	0
80° — 12 min.	0	95.0	4.5	0.5	0	0	0	0
80° — 15 min.	0	—	—	—	—	—	—	—
Virus in buffer mixture of pH 5.2								
Control (unheated)	218	—	—	—	—	—	—	—
75° — 10 min.	193	—	15.0	21.5	54.5	6.5	2.5	0
80° — 10 min.	36	—	32.5	34.0	30.5	2.0	1.0	0
85° — 10 min.	0	48.0	11.0	22.5	9.5	4.0	5.0	0
90° — 10 min.	0	—	—	—	—	—	—	—

* Total number of necrotic lesions produced on 30 leaves of *Nicotiana glutinosa* plants.

paratively higher temperatures upon the tobacco mosaic virus is attributable to their action in breaking the virus particles as well as to their thermal effect upon the chemical constituents of the virus which consist of nucleoprotein.

Summary

The electron micrographs of chemically purified potato virus X, mottle strain, demonstrated that the virus particles were sinuous rods or filaments. Fifty-nine per cent and 74 per cent of the virus particles obtained from affected tomato and tobacco plants, espectivelyr were 400-500 mμ long and about 10 mμ wide. Most of the virus particles were from 425 to 450 mμ in length.

A suspension of chemically purified tobacco mosaic virus was heated on the collodion films on specimen screens at 60°, 70°, 80°, 90°, and 95°C for varying lengths of time. There was a slight decline in the infectivity of the virus in the suspension which was heated at 70° for 30 or 40 minutes ; on the other hand, when viewed in the electron microscope, it was found that virus particles shorter than 230 mμ increased and those equal to or longer than 280 mμ were reduced in number. Heating of the virus suspension at 80° for 5 minutes apparently caused no effect upon the infectivity of the virus but resulted in the aggregation of the latter into networks, whereas 10 minutes' heating caused a remarkable decrease in virus activity and corresponding increase of virus less than 230 mμ in length and small globular deposits. The virus suspensions which were exposed to heat at 90° or 95° for 10 minutes showed coagulated amorphous masses and small globular deposits, no rod-shaped virus particles being found, while heating at 90° for 10 minutes rendered the virus suspension entirely noninfectious.

In another series of experiments the virus in buffer mixture of pH 7.0 was heated by immersing a test tube which contained the virus suspension into the water bath at the desired temperature. The infectivity of virus suspension began to diminish when heated at 75° for 10 minutes and was entirely lost at 80° for 10 minutes. When the virus suspensions were heated at 80° for 5, 8, 10, 12, and 15 minutes, the electron micrographs obtained demonstrated that prolonged heat treatment was accompanied by an increase in the number of comparatively shorter virus rods which

were eventually replaced by globular particles.

In short, infectivity assay and electron microscopic examination of the virus suspension exposed to comparatively higher temperatures showed that both infectivity and number of virus particles longer than 230 mμ were reduced with the rising of temperature and prolongation of exposure time.

Literature cited

1) BAWDEN, F. C.: Plant viruses and virus diseases. 3rd ed. Waltham, 1950.
2) ——— and N. W. PIRIE: Liquid crystalline preparations of potato virus 'X'. Brit. Jour. Exp. Path. 19 : 66–82. 1938.
3) BODE, O. und E. KÖHLER: Elektronenmikroskopische Untersuchungen am Kartoffel-X- und Tabakmosaikvirus. Z. Naturf. 7b 11 : 598–600. 1952.
4) FUKUSHI, T.: Advances in the study of plant virus diseases. (in Japanese) Nogyo-Engei [Agric. and Hort.] 26 : 9–13. 1951.
5) ———: Plant viruses. (in Jap.) Tokyo, 1952.
6) ——— and E. SHIKATA: Electron microscope studies on plant viruses. (in Jap.) Ann. Phytop. Soc. Japan 16 (3-4) : 173 (abstract). 1952.
7) ——— and ———: Visualization of viruses. (in Jap.) ibid. 17 (1) : 35 (abstract) 1952.
8) ——— and ———: Change of tobacco mosaic virus at higher temperature. (in Jap.) ibid. 18 (1-2) : 61 (abstract). 1953. (Also in Virus 3 : 208. 1953).
9) HIDAKA, J. and H. MURANO: On the shape and size of tobacco mosaic virus. (in Jap.) ibid. 16 (1) : 32–33 (abstract). 1952.
10) ———, K. KIRIYAMA and Y. NISHIJIMA: Inactivation of tobacco mosaic virus. (in Jap.) ibid. 17 (1) : 45 (abstract). 1952.
11) ——— and ———: Inactivation of tobacco mosaic virus. (in Jap.) Virus 3 (1) : 39–50. 1953.
12) HIGASHI, N.: Virus research by means of the electron microscope. (in Jap.) Kagaku [Science] 19 (10) : 434–439. 1949. (Also in Denshi-kenbikyo [Electron microscope] 1 (1) : 6–11. 1950.
13) KAUSCHE, G. A., E. PFANKUCH und H. RUSKA: Die Sichtbarmachung von pflanzlichem Virus im Übermikroskop. Naturwis. 27 : 292–299. 1939.
14) ———, ——— und ———: Beobachtungen über Schall und Ultraschalleinwirkungen am Protein des Tabakmosaikvirus. ibid. 29 : 573–574. 1941.
15) LORING, H. S.: Properties of the latent mosaic virus protein. Jour. Biol. Chem. 126 : 455–478. 1938.
16) OSTER, G.: Studies on the sonic treatment of tobacco mosaic virus. Jour. Gen. Physiol. 31 : 89–102. 1947.

17) OSTER, G., C. A. KNIGHT and W. M. STANLEY: Electron microscope studies of Dahlem, Rothamsted and Princeton samples of tobacco mosaic virus. Arch. Biochem. 15 : 279-288. 1947.

18) RAWLINS, T. E., C. ROBERTS and N. M. UTECH: On electron microscope study of tobacco mosaic virus at different stages of infection. Amer. Jour. Bot. 33 : 356-363. 1946.

19) RUSKA, H.: Die Elektronenmikroskopie in der Virusforschung. in DOERR u. HALLAUER Handbuch der Virusforschung. II Ergänzungsband : 221-417. 1950.

20) SIGURGEIRSSON, T. and W. M. STANLEY: Electron microscope studies on tobacco mosaic virus. Phytop. 37 : 26-38. 1947.

21) SMITH, K. M.: Recent advances in the study of plant viruses. 2nd ed. London, 1951.

22. TAKAHASHI, W. N.: The effect of pH on the linear aggregation of tobacco mosaic virus. Amer. Jour. Bot. 36 : 642-645. 1949.

23) ———— and T. E. RAWLINS: An electron microscope study of two strains of potato virus X. ibid. 33 : 740-742. 1946.

24) ———— and ————: An electron microscope study of tobacco mosaic virus extracted from pulp and juice, after various periods of infection. Phytop. 38 : 279-282. 1948.

25) ———— and ————: The length of tobacco mosaic virus particles from juice and from leaf residue. ibid. 39 : 672-675. 1949.

26) TERADA, M. and K. FUKAI: Microbes. Atlas of Electron Micrographs. I. Kyoto, 1952.

27) TODD, C.: On the electrical behaviour of the bacteriophage. Brit. Jour. Exp. Path. 8 : 369-376. 1927.

28) WILLIAMS, R. C. and R. W. G. WYCKOFF: The thickness of electron microscope objects. Jour. Appl. Phys. 15 : 712-716. 1944.

29) ———— and ————: Electron shadow micrography of the tobacco mosaic virus protein. Sci. 101 : 594-596. 1945.

30) ———— and ————: Application of metallic shadow-casting to microscopy. Jour. Appl. Phys. 17 : 23-33. 1946.

31) WYCKOFF, R. W. G.: Electron microscopy. New York and London, 1949.

Explanation of illustrations

Pl. I Electron micrographs of potato virus X.

Figs. 1 and 2 Virus filaments isolated from affected tomato and tobacco plants, respectively. (Chromium-shadowing). × 40,000 [Electron micrographs by H. YOTSUMOTO].

Pl. II Electron micrographs of tobacco mosaic virus heated at 70°C for various lengths of time. (Virus in dialyzed suspension) Chromium-shadowing, × 25,000 [Electron micrographs by S. TAKAHASHI with the exception of Fig. 1]

Fig. 1, Unheated control [Electron micrograph by H. TSUCHIKURA]

Fig. 2, Virus rods heated at 70° for 5 minutes; Fig. 3, Virus rods heated at 70° for 10 minutes; Fig. 4, Virus rods heated at 70° for 20 minutes; Fig. 5, Virus particles heated at 70° for 30 minutes; Fig. 6, Virus particles heated at 70° for 40 minutes.

Pl. III Electron micrographs of tobacco mosaic virus exposed to higher temperatures. (Virus in dialyzed suspension) Chromium-shadowing, × 25,000 [Electron micrographs by S. TAKAHASHI]

Fig. 7, Virus rods heated at 80° for 5 minutes; Fig. 8, Virus particles heated at 80° for 10 minutes; Fig. 9, Virus particles heated at 80° for 20 minutes, showing virus rods which were apparently divided into small segments.

Fig. 10, Small globular deposits which were revealed in the virus suspension heated at 80° for 20 minutes.

Fig. 11, Small globular deposits found in the virus suspension heated at 80° for 30 minutes.

Fig. 12, Globular deposits which were found in the virus suspension heated at 90° for 10 minutes.

Pl. IV Fig. 13, Electron micrograph of tobacco mosaic virus heated at 95° for 10 minutes.

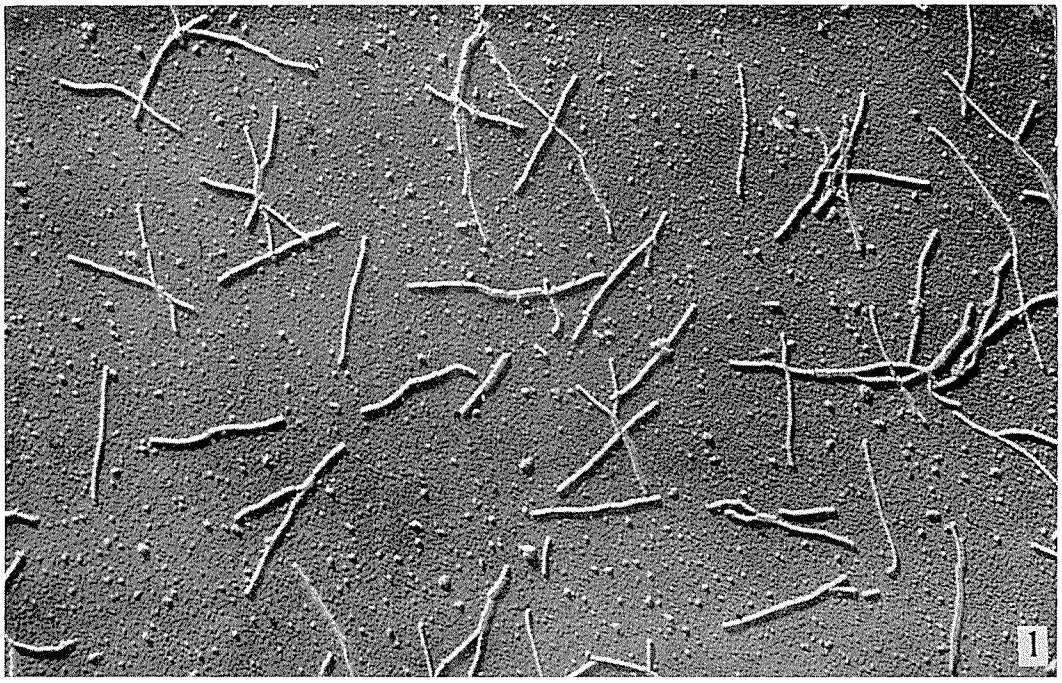
Figs. 14–18, Electron micrographs of tobacco mosaic virus heated at various temperatures. (Virus particles dispersed in phosphate buffer of pH 7.0) Chromium-shadowing, × 25,000

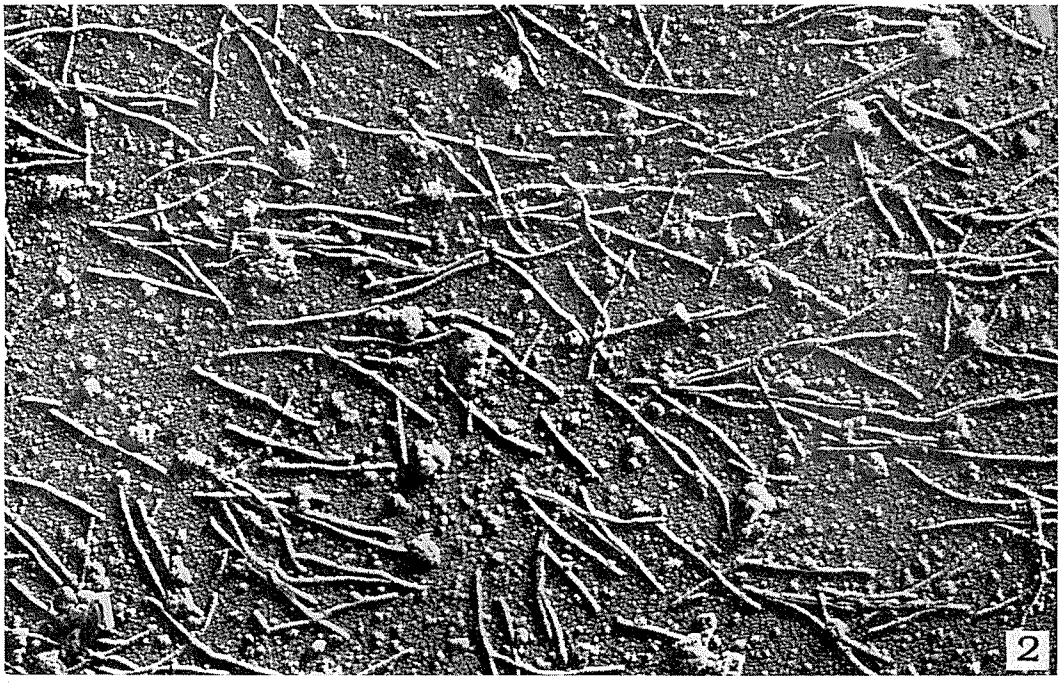
Fig. 14, Unheated control; Fig. 15, Virus rods heated at 70° for 10 minutes; Fig. 16, Virus rods heated at 75° for 10 minutes; Fig. 17, Virus suspension heated at 80° for 10 minutes showing a few virus rods scattering among small globular particles; Fig. 18, Virus

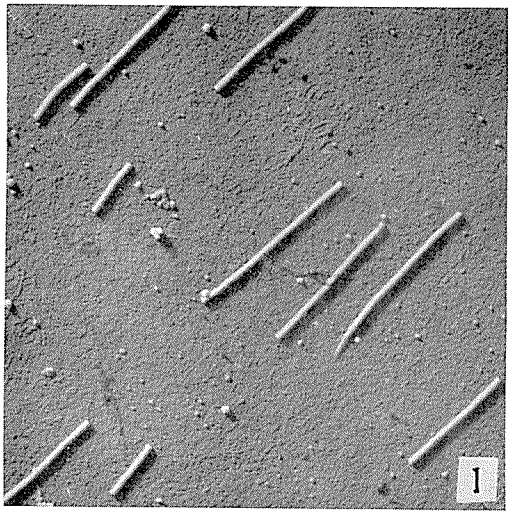
suspension heated at 85° for 10 minutes showing small globular particles.

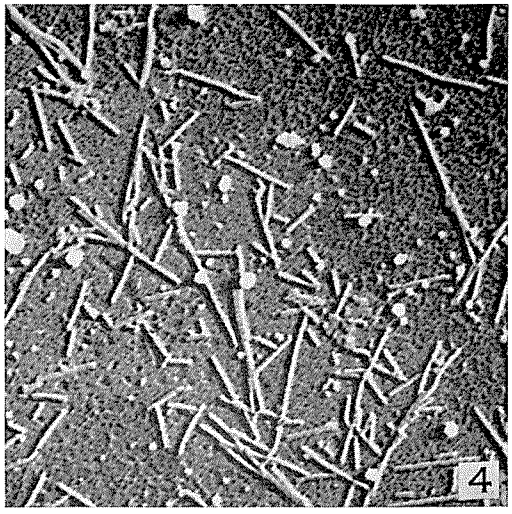
Pl. V Electron micrographs of tobacco mosaic virus heated at 80° for different lengths of time. (Virus particles dispersed in phosphate buffer of pH 7.0) Chromium-shadowing, × 25,000

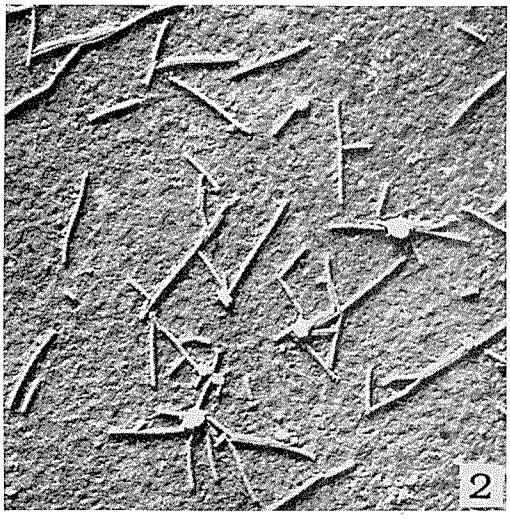
Fig. 19, Virus suspension heated at 80° for 5 minutes; Fig. 20, Ditto heated at 80° for 8 minutes; Fig. 21, Ditto heated at 80° for 10 minutes; Fig. 22, Ditto heated at 80° for 12 minutes; Fig. 23, Ditto heated at 80° for 15 minutes; Fig. 24, Ditto heated at 80° for 20 minutes.

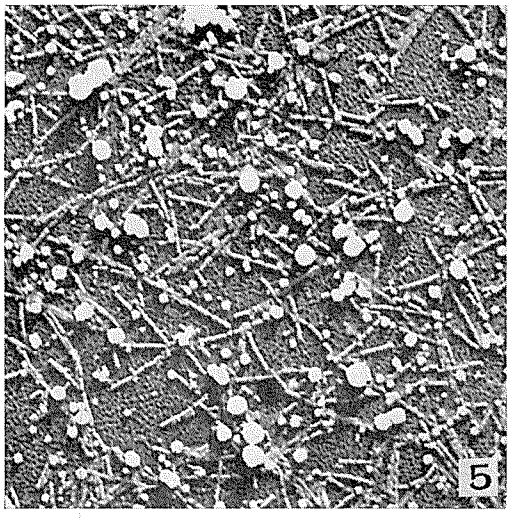

1

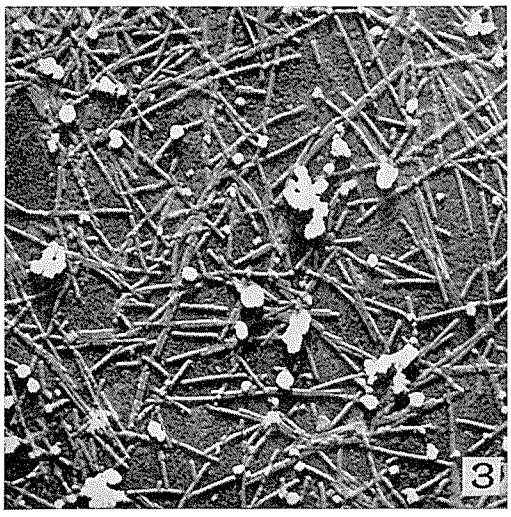

2

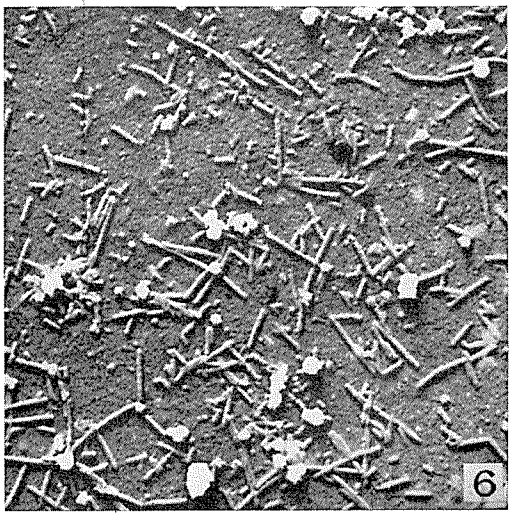

1

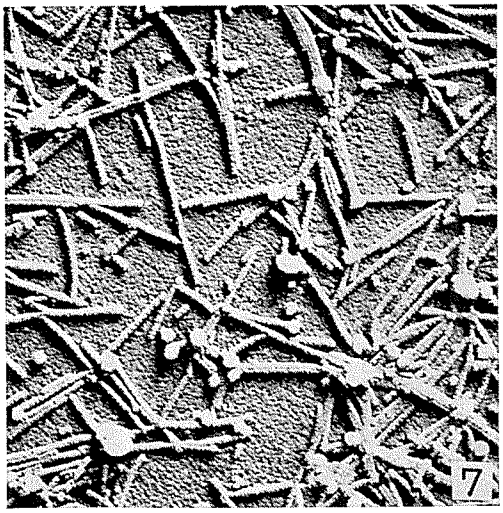

4

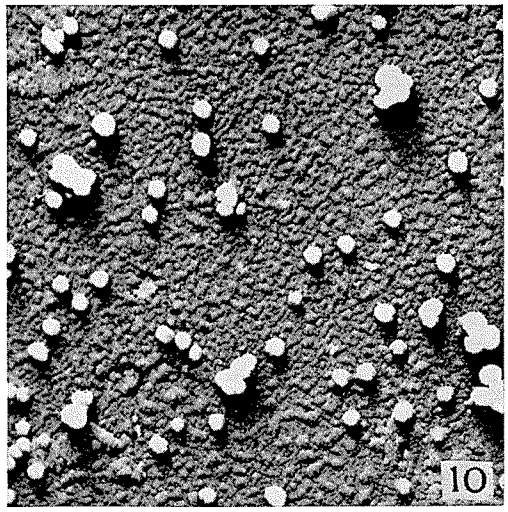

2

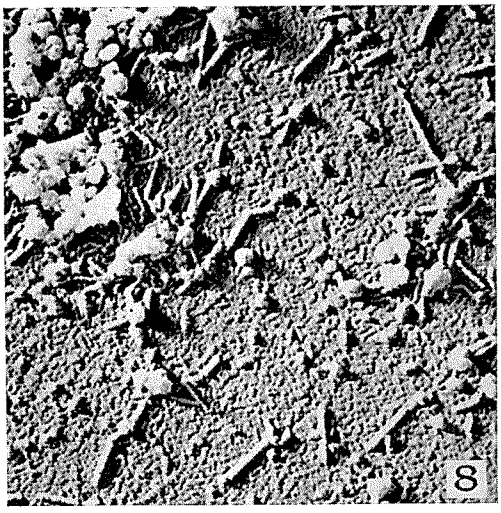

5


3

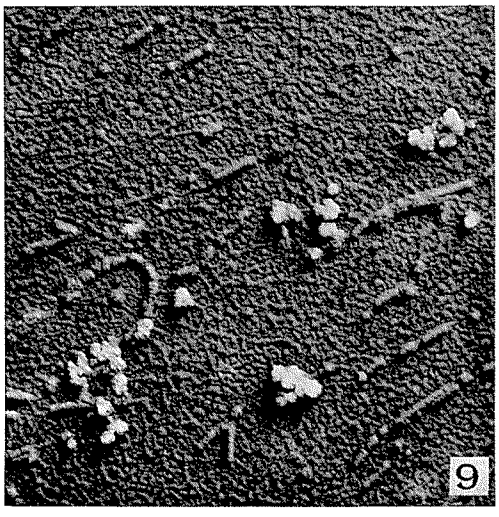

6

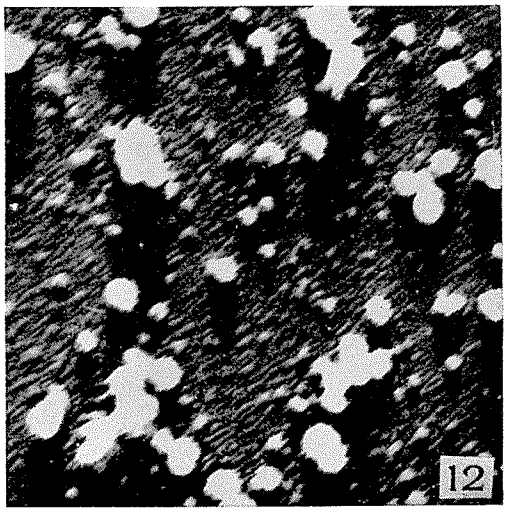

7

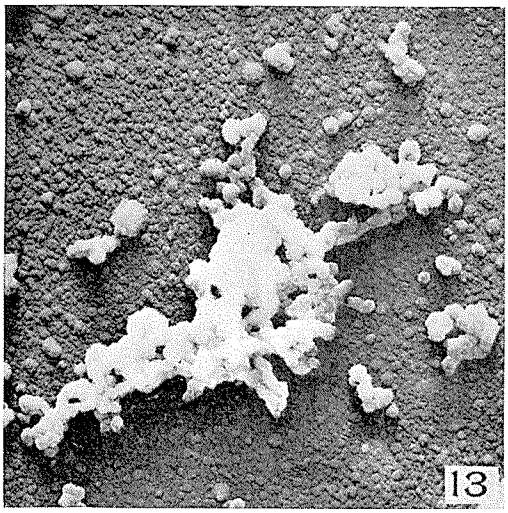

10

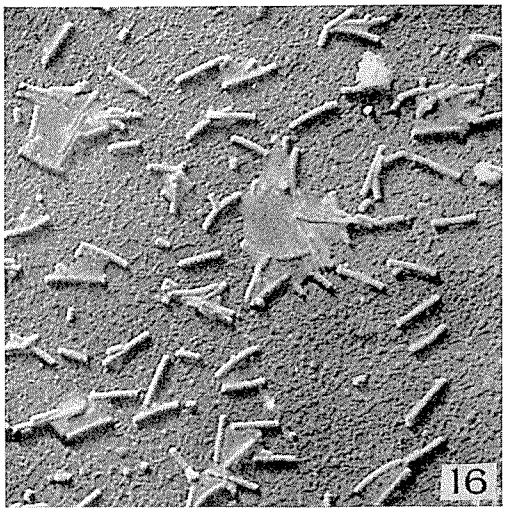

8

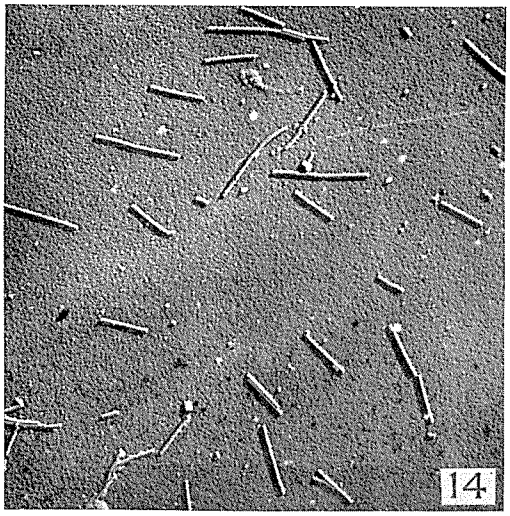

11

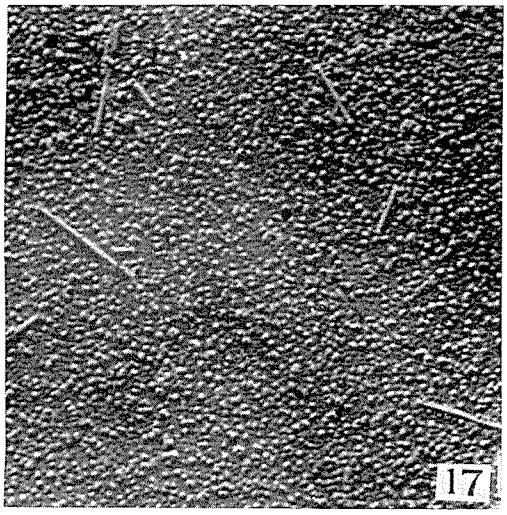

9

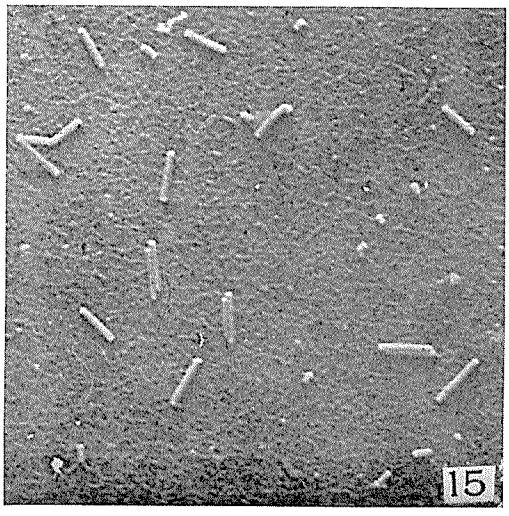

12

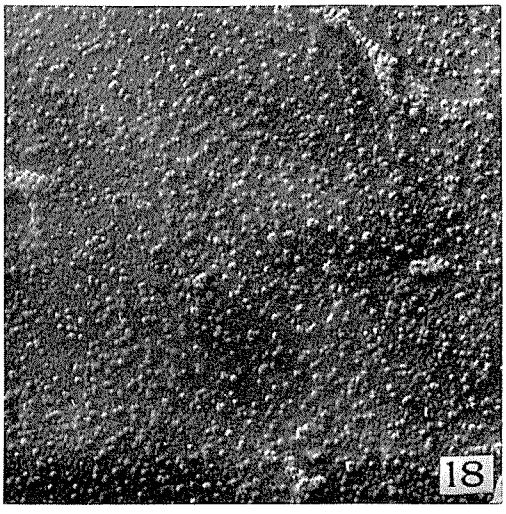

13

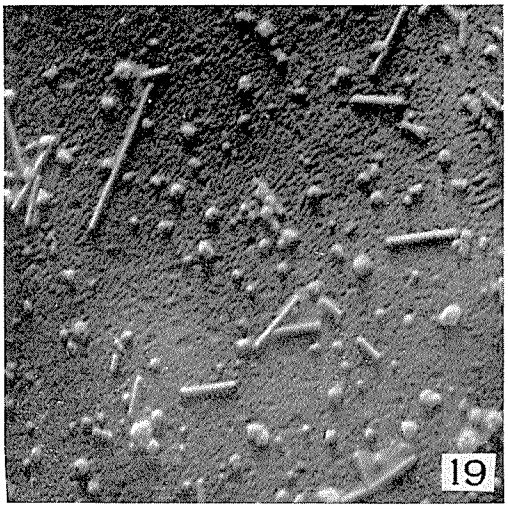

16

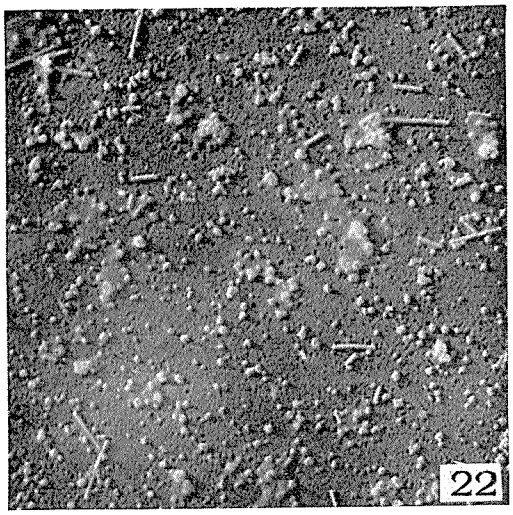

14

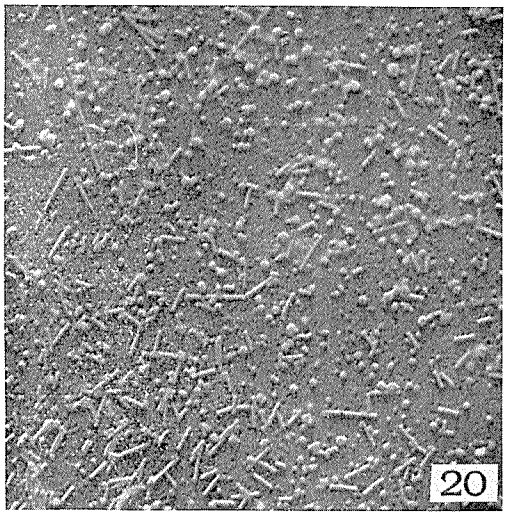

17

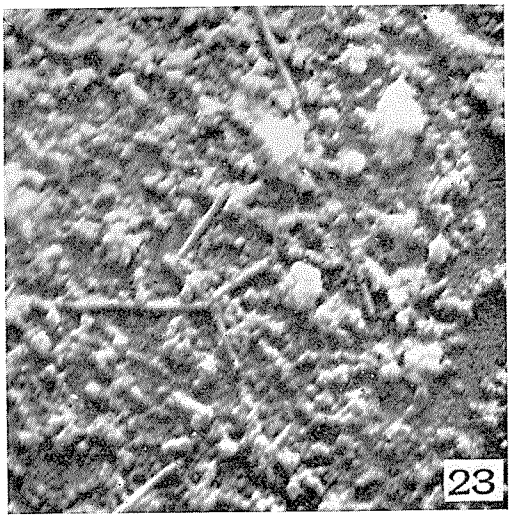

15

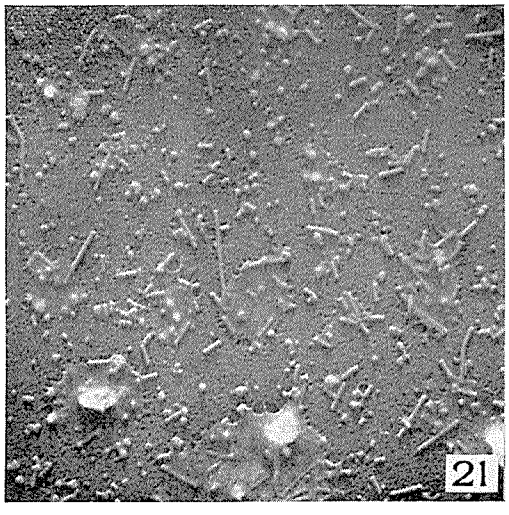

18

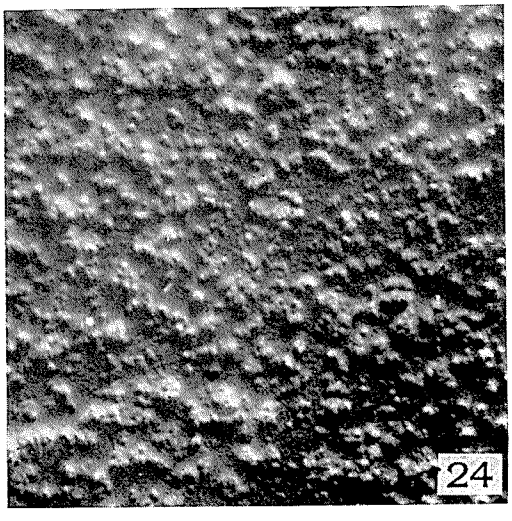

19


22


20


23


21


24