

Classification of Elongated Plant Viruses on the Basis of Particle Morphology

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Shape, diameter, and length of particles are morphological characteristics of elongated plant viruses. The length is the most useful feature for characterizing a distinct virus. When the exudate method is used for electron microscopic preparations, 60-80 % of all measured particles usually show specific favored lengths. These appear as maxima in the distribution curves. The characteristic length value, calculated as the arithmetical mean from the main maximum is called "normal length." The results of various authors allow the supposition that particles of normal length correspond to the infectious units.

Based upon the results of comparative measurements, a number of elongated viruses are placed in twelve groups according to their normal lengths. Viruses belonging to one group and representing distinct species are sometimes related serologically, sometimes not. Additional characteristics, such as diameter, shape, thermal inactivation point, and mode of transmission, show correlations within each group and suggest higher systematic units into which viruses with different normal lengths can be combined.

INTRODUCTION

The existing systems according to which plant viruses have been named and catalogued by several authors (e.g., Johnson and Hoggan, 1935; Smith, 1937; Holmes, 1939; Hansen, 1956) suffer from the fact that they are based chiefly on secondary characteristics such as symptomatology, host plants, and mode of transmission. As properties of the viruses themselves could not be taken as a basis, these attempts at cataloguing led to artificial systems which have little or no regard for natural relationships. Criticisms of the older systems of classification have been made by Andrewes (1955) and Bawden (1955). As a basis for further proposals on classification, the Virus Subcommittee of the Inter-

national Nomenclature Committee (1950) suggested eight criteria according to which a system for viruses ought to be formed. The first and most important characteristic noted was "Morphology and Methods of Reproduction."

Plant viruses can be divided arbitrarily in two groups according to the morphology of the particles: viruses with nearly spherical shape, and those of an elongated form. In this paper an attempt has been made to classify elongated viruses on a morphological basis. Special attention has been paid to the method by which reproducible values for the length of virus particles can be obtained.

METHODS OF PREPARATION AND MEASUREMENT

Whereas viruses of spherical shape can be prepared for electron microscopy best by chemical and physical methods, these methods still have disadvantages as far as elongated viruses are concerned. Such purification procedures, if at all successful, generally change the particles in such a way that it is difficult to determine exact lengths. However, the density-gradient centrifugation techniques of Brakke (1953) have opened new possibilities here.

With Johnson's exudate method (1951), satisfactory results have been obtained in the preparation and measurement of elongated viruses (Brandes and Paul, 1957; and footnotes to Table 1). The particles in most cases can be measured in spite of the contamination of the preparations by cell fragments. In some cases the exudate method cannot be applied because the morphology of the plants (e.g., cactus plants) does not permit the successful use of the apparatus. We were able to overcome these difficulties by a procedure called "the dipping method" (Brandes, 1957). According to Gold *et al.* (1957b), spraying diluted unpurified homogenates on the collodion film is a suitable method for obtaining uniform particles. The photographs of the particles used for comparative studies, cited in Table 1 (a), were taken with an electrostatic electron microscope (AEG/Zeiss; EM 8/2) at a fixed magnification ($\times 5320$). The measurements were made at a total magnification of 40,000.

EXPERIMENTAL RESULTS AND STATISTICAL ANALYSIS OF THE "NORMAL LENGTH"

The virus particles obtained by the exudate method are of great uniformity. In general 60–80% of them show favored lengths. In the histogram of measured particles those of favored lengths appear as a

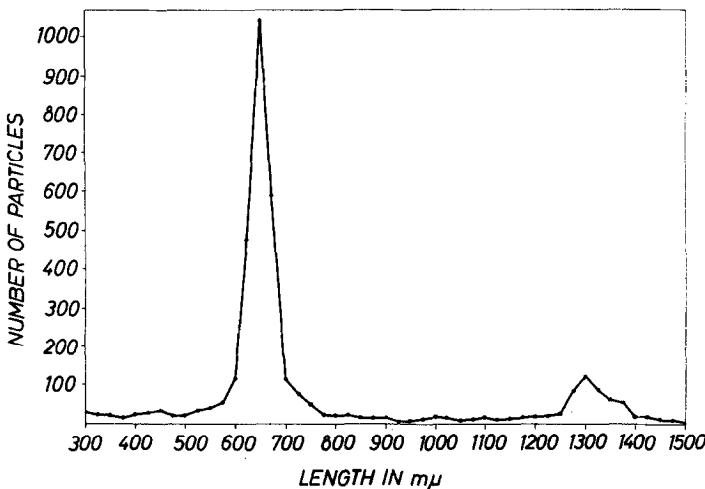


FIG. 1. Distribution curve of particle lengths of potato virus S, potato virus M, and carnation latent virus.

main maximum and occasionally as one or two smaller maxima, representing half and double lengths (see Fig. 1). On the other hand, particles not belonging to the maxima are spread along the total length scale in nearly equal frequency at all points. The percentage of particles showing such favored lengths may vary. There are indications that the age of the infected host tissue is of importance (Bercks and Querfurth, 1956). Nevertheless the maxima are distinct if one measures a sufficient number of particles. In general 100 units are sufficient for the calculation of an average value.

The arithmetical mean calculated from the main maximum has been designated "normal length" (NL) (Wetter and Brandes, 1956; Bode and Paul, 1956). It is characteristic for each type of elongated virus. The NL of a given virus prepared from different sources has always been found invariable except for the errors caused by electron microscopy and the technique of measurement. It may be stressed that the absolute length of particles is less important for the determination of NL than are the relative differences between viruses under identical conditions.

The distribution curve of particle lengths (Fig. 1) can be separated arbitrarily into two fractions: the specific lengths concentrated within the peaks and the unspecified ones bordering the peaks. These bi- or polymodal distributions do not allow an exact statistical treatment

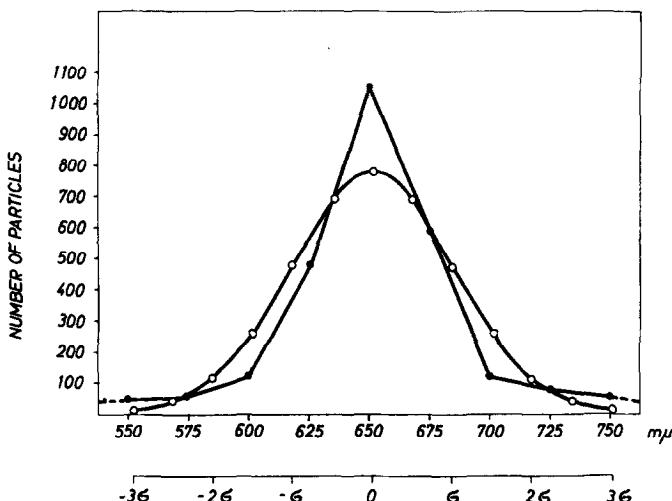


FIG. 2. The distribution curve of the main maximum from Fig. 1 (●) in comparison with the corresponding normal distribution curve (○).

as far as we know. It is obvious, however, that a representative mean value of the length can be calculated only if unspecific lengths are neglected. The difficulty is based on the fact that there is apparently no mathematical method for the delimitation of the main maximum. Usually, we have considered for calculation only those particles which exceed the level constituting the unspecific lengths in the histogram.

In order to find out whether the distribution of lengths in the delimited main maximum corresponds to a normal distribution, the lengths for a large number of particles of the potato viruses S and M, and of the carnation latent virus have been represented in one curve (Fig. 1). The appropriate normal distribution for the main maximum has been calculated (Fig. 2). Among the three serologically related viruses no significant differences in length could be found (Brandes *et al.*, 1959). The particles exceeding the level of unspecific lengths are distributed as follows:

Class (x_i)	550	575	600	625	650	675	700	725	750	$m\mu$
Frequeney (z_i)	43	57	117	476	1045	588	115	79	51	
$N = 2571$										

The calculation of the mean (\bar{x}), of the standard deviation (s) and the excess (E) gives the following results: $\bar{x} = 652.0 \text{ m}\mu$; $s = \pm 33.1$

$m\mu$; $E = +1.75$. For the calculation of the excess equation (1) has been used (Weber, 1956):

$$E = \frac{\sum z_i(x_i - \bar{x})^4}{N \cdot s^4} - 3 \quad (1)$$

The distribution of the lengths does not fit the appropriate normal distribution, as the modal class x_0 is excessively favored, a fact proved by a positive value for the excess.

As to the variability expressed by the distribution curve of the main maximum, one must consider the possibility that the curve might combine two different components: (1) true variability of virus particles; (2) variability due to errors. The latter include inevitable changes in the particles that occur from the beginning of the exudation process until photography in the electron microscope, as well as from measuring errors. It can be assumed that the distribution of the errors corresponds to a normal distribution and that this fact therefore might feign a normal distribution of lengths if only a few particles were to be measured. However, in our study the curve shows an excess which is more pronounced if one includes in the calculation some more classes below class 550 and above class 750. Although the length of the particles may be variable, it can be concluded that there is a more frequent modal length, whereas shorter and longer particles immediately neighboring the modal class are fewer than expected in a normal distribution.

The most logical explanation is, to our mind, that the distribution curve actually is composed of two different fractions: (1) the specific lengths concentrated within the peaks, the distribution of which indicates a high homogeneity of the virus particles, and (2) the unspecific lengths distributed over the remaining range of the length scale. It is not known whether the latter particles are artifacts or incomplete forms. However, the increase of their number obtained by preparation methods different from the exudate method indicates that they may be artifacts. As to the particles of specific length within the main maximum it seems conceivable that they represent a fundamental unit of a virus. One can imagine that the variability of this unit as a chemical entity shows significant differences from common biological variability.

Since the distribution of lengths does not correspond to a normal distribution and since the delimitation of the main maximum is more or less arbitrary, no exact value for the standard deviation can be calculated. On the other hand, the arithmetical mean can be calculated

exactly enough, whatever classes are chosen for the delimitation of the main maximum, as the following values show:

Classes:	625-675,	600-700,	575-725,	550-750,	525-775,	500-800	$m\mu$
Mean:	651.3	651.1	651.7	652.0	651.4	651.2	$m\mu$

Another method for determining central tendency is the calculation of the mode (Brandes and Paul, 1957). In our example this results in a value ($651.4\text{ m}\mu$) very similar to the arithmetical mean. The mode was calculated from equation (2) (Weber, 1956):

$$\text{Mode} = x_0 - \frac{d}{2} + \frac{n_0 - n_{-1}}{2n_0 - n_{-1} - n_{+1}} \cdot d \quad (2)$$

d = class interval; n_0 , n_{-1} , n_{+1} = frequencies of the classes x_0 , x_{-1} , x_{+1} .

NL AND THE INFECTIOUS UNIT

It seems reasonable to assume that the particles of favored length are identical with the agent inciting the corresponding disease, since the characteristic particles are present in diseased plants only and not in healthy ones. This identity has been established for a few elongated viruses. Mundry (1958) found that the shortest infectious unit of beet yellows virus was in best agreement with the NL (about $1250\text{ m}\mu$) determined by Brandes and Zimmer (1955). Brakke and Staples (1958) showed that particles of wheat streak mosaic virus are infectious only if longer than $650\text{ m}\mu$. From the length distribution curve in this paper one can calculate a mean of about $700\text{ m}\mu$. This value has been confirmed by Brandes (1959). In the case of tobacco mosaic virus (TMV) there have been numerous papers since the studies of Lauffer (1943) and Schramm (1943) indicating that the smallest infectious unit is about $300\text{ m}\mu$ in length. Moreover, according to Gierer (1958), the infectious unit of the ribonucleic acid of TMV corresponds to the total RNA-content of the $300\text{-m}\mu$ rod. Concerning the length of potato stem mottle virus (tobacco rattle virus) a peculiar feature was observed by Paul and Bode (1955). Two maxima of almost equal size were found to have no simple proportion to one another ($70\text{ m}\mu$ and $180\text{ m}\mu$). Recently B. D. Harrison and H. L. Nixon (personal communication, 1958) found the shorter particles to be noninfectious.

CLASSIFICATION ON THE BASIS OF NL

Elongated viruses have been measured by several authors. The results are summarized in Table 1 (see Fig. 3). In *a* are listed those viruses,

TABLE 1
ARRANGEMENT OF ELONGATED PLANT VIRUSES ACCORDING TO NORMAL LENGTH, SHAPE, DIAMETER, MODE OF TRANSMISSION, AND THERMAL INACTIVATION POINT^a

Group	1	2	3	4	5	6
NL (mμ) ~	130	180	300	480	515	580
Shape	Rigid rods			Flexible threads		
Diameter (mμ) ~	20		15	10-11		
Transmission	Sap; soil		Sap	Sap; (aphids?)		
TIP ~	—	75-80°C	85-95°C	60-75°C		
a	Barley stripe mosaic virus (1)	Potato stem mottle virus (3)	+ Tobacco mosaic virus (4) + Cowpea virus (5)	White clover mosaic virus (8)	Potato virus X (9) Cactus virus 1 (10)	Potato aucuba mosaic virus (11)
b	Soil - borne wheat mosaic virus (2)		+ Cucumber green mottle virus (6) <i>Odontoglossum</i> ring spot virus (7)	<i>Cymbidium</i> mosaic virus (7)		

^a +, O, Δ Indicate serological relationship within groups 3, 8, and 11, respectively. Numbers in parentheses refer to footnotes as follows:

(1) Gold *et al.* (1954): 130 × 30 mμ. Our measurements (Brandes, 1959) gave an NL of 126 mμ, but the diameter of the particles was only about 20 mμ when compared with TMV and potato stem mottle virus.

(2) Gold *et al.* (1957).

(3) Paul and Bode (1955) found two frequent lengths: 70 and 180 mμ. The values of Harrison and Nixon (personal communication) are in good accordance. These authors could show that the shorter particles are noninfectious. Therefore, 180 mμ must be taken as NL. Potato stem mottle virus also includes tobacco rattle virus and soil-borne potato viruses found in the United States (Walkinshaw and Larson, 1958; Oswald and Bowman, 1958). The morphological data are apparently the same; furthermore, serological relationship has been established.

(4) A great number of papers exist; 300 mμ is especially the result of detailed measurements by Williams and Steere (1951). TMV often has been used as a standard in our laboratory.

(5) The cowpea virus was kindly supplied by F. C. Bawden.

(6) A detailed study on this subject is that of Knight (1955).

(7) Jensen and Gold (1951); Newton and Rosberg (1952); Gold and Jensen (1953); Murakishi (1958).

(8) Brandes and Quantz (1957).

(9) Bode and Paul (1955).

(10) With the aid of the dipping method we found two different viruses in several species of cactus plants (Brandes and Uschdorff, unpublished). Cactus virus 1 possibly is identical with a virus described by Amelunxen (1958).

(11) Paul and Bode (1956b).

(Table continued on next page)

TABLE 1—Continued

Group	7	8	9	10	11	12
NL ($m\mu$) ^a	620	650	700	730	750	1250
Shape	Rods, rigid to slightly flexible			Flexible threads		Very flexible threads
Diameter ($m\mu$) ~	12-13		12-13			10
Trans-mission	Aphids; sap		Mites; sap	Aphids; sap		Aphids; (sap)
TIP ~	65-75°C		—	—	50-60°C	—
a	Wisconsin pea streak virus (12)	Red clover vein mosaic virus (12) ○ Carnation latent virus (13) ○ Potato virus S (13) ○ Potato virus M (13) Cactus virus 2 (10)	Wheat streak mosaic virus (16)	Beet streak mosaic virus (16) Potato virus A (18) Potato virus Y (18) Tobacco etch virus (19) Henbane mosaic virus (19)	Beet yellows virus (25) Potato virus A (18) Potato virus Y (18) Tobacco etch virus (20) Pea mosaic virus (20) Soybean mosaic virus (20) Turnip mosaic virus (21) Cocksfoot streak virus (19) Lettuce mosaic virus (22)	Beet common mosaic virus (20) Δ Bean yellow mosaic virus (20) Δ Pea mosaic virus (20) Soybean mosaic virus (20)

b	"Hop virus" (14) "Gledidolus virus" (14)	"Poplar virus" (14) Tulip-breaking virus (15)	Sugar cane mosaic virus (24)
(12) Wetter <i>et al.</i> (1959); Wetter and Quantz (1958); Brandes and Quantz (1957).			
(13) Brandes <i>et al.</i> (1959).			
(14) Electron microscopic studies of these viruses are going on in our laboratory. The hop virus is being studied by K. Nuber (Landwirtschaftliche Hochschule, Hohenheim).			
(15) De Bruyn Ouboter <i>et al.</i> (1951).			
(16) Gold <i>et al.</i> (1957a); Brandes (1959).			
(17) Zimmer and Brandes (1956).			
(18) Bode and Paul (1956; Paul and Bode (1956a); Brandes and Paul (1957).			
(19) Brandes (1959).			
(20) These four legume viruses all have the same size (Brandes and Quantz, 1955; Quantz, 1958). Serological relationship has been estab-			
lished between bean common mosaic virus and bean yellow mosaic virus (Beensier and van der Want, 1951; Borek, personal communication) and between bean yellow mosaic virus and pea mosaic virus (Goodchild, 1953).			
(21) Bode and Brandes (1958).			
(22) Couch and Gold (1954): $746 \times 22 \text{ m}\mu$. Our determination of the NL revealed the same value ($747 \text{ m}\mu$), but measurements in comparison with TMV and other viruses revealed a diameter of about $12-13 \text{ m}\mu$ (Brandes, 1959).			
(23) This virus has been described and suspected of being related to sugar cane mosaic virus by Grancini (1957). It has been measured in our laboratory (Brandes, 1959).			
(24) Gold <i>et al.</i> (1957a).			
(25) Brandes and Zimmer (1955); Burghardt and Brandes (1957).			

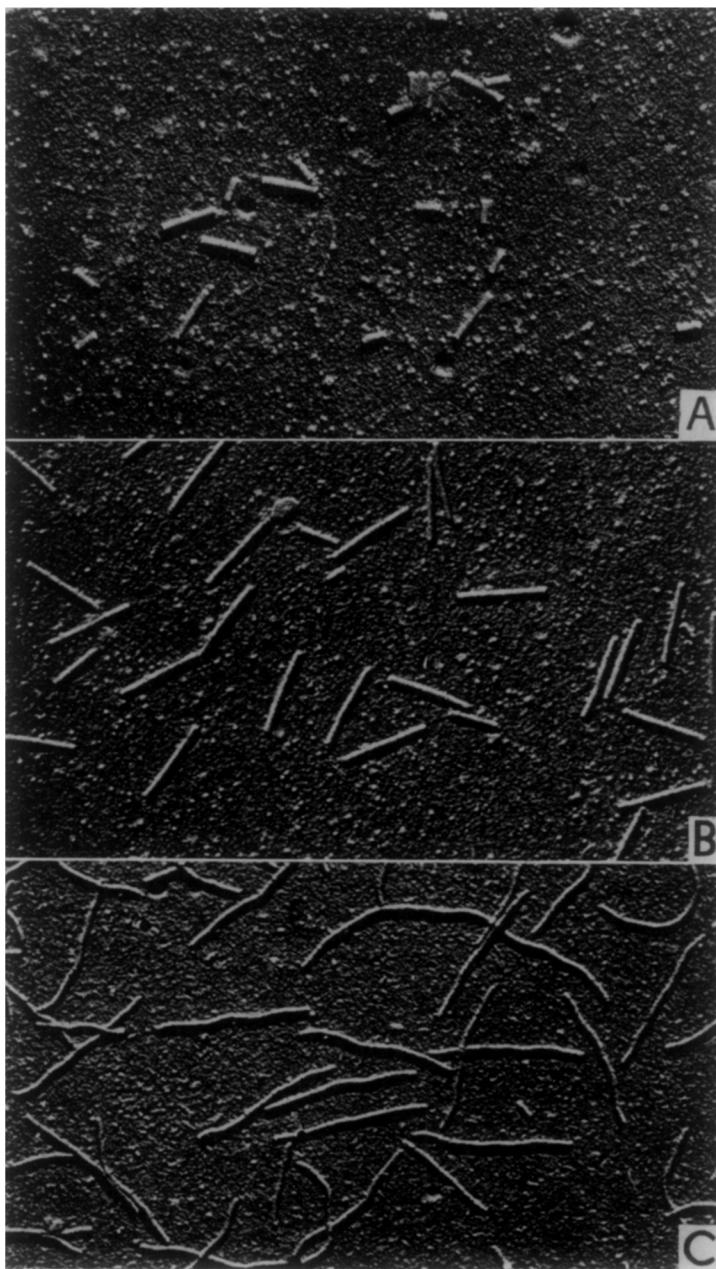


FIG. 3. A, B AND C

FIG. 3. Virus particles representing shapes designated in Table 1. Magnification: $\times 40,000$; palladium-shadowed. A. Potato stem mottle virus. B. Tobacco mosaic virus. C. Potato virus X.

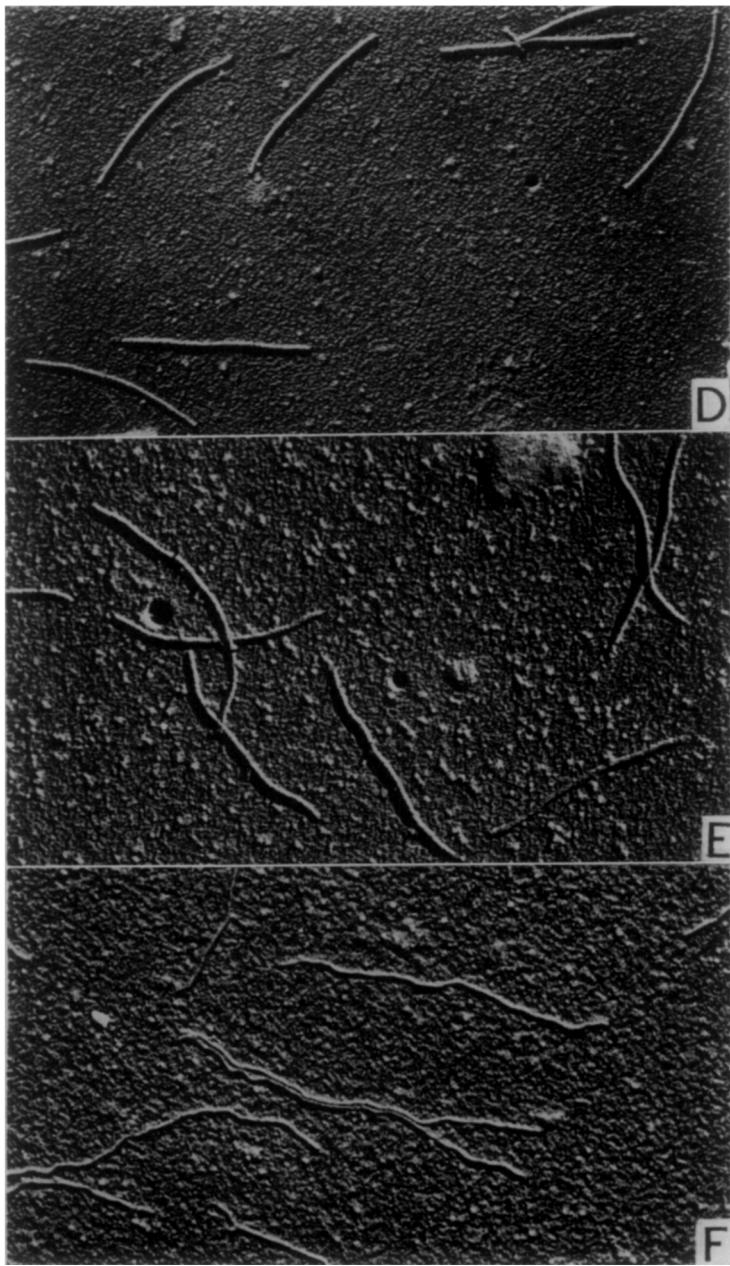


FIG. 3. D, E AND F

D. Wisconsin pea streak virus. E. Potato virus Y. F. Beet yellows virus.

comparative measurements of which were done in our laboratory using the exudate or the dipping method. In *b* are placed other viruses which are thought to belong to corresponding groups. "Common names" from the *Review of Applied Mycology* (1957), Supplement Volume 35, have been used. The index numbers in parentheses in the table refer to the appended footnotes in which the original papers are quoted. About forty viruses are classified into twelve different groups according to their NL's (Table 1). Probably the number of these groups will increase with the morphological description of additional viruses.

ROLE OF ADDITIONAL CHARACTERISTICS AND DISCUSSION OF THEIR SYSTEMATIC ASPECTS

Shape, diameter, mode of transmission, thermal inactivation point (TIP), and serological relationship also have been used for forming the new system.

According to the *shape* of the virus particles one may differentiate between four different forms: (1) rigid rods (groups 1-3); (2) rods, rigid to slightly flexible (groups 7-9); (3) flexible threads (groups 4-6, 10, 11); (4) very flexible threads (group 12). Arrangement of the particles according to shape does not always fit the order according to NL, because a column of rodlike viruses falls between two columns of flexible threads in the range of the length scale. Viruses belonging to the groups 4-6 are separated from the longer flexible ones by other qualities, so that the shape of the virus particles alone is not a basic characteristic unless the connection to NL and diameter is considered.

Unfortunately it is much more difficult to determine the *diameter* of particles as exactly as the length by electron microscopy. Significant differences exist only between viruses with diameters of about 20 m μ (groups 1, 2), of about 15 m μ (group 3), and of about 10-13 m μ (groups 4-11). Possibly the viruses of groups 4-6 and 11 are a little thinner than the viruses of groups 7-10. For the exact determination of the diameter perhaps other methods than electron microscopy will be more suitable. X-Ray data indicate that the diameter of the particles of cucumber green mottle virus is about 5 Å less than that of TMV (Franklin, 1956), a difference which cannot be proved by electron microscopy.

The data for mode of transmission and TIP (given in Table 1) are taken partly from Smith's (1957) *Textbook of Plant Virus Diseases*. For TIP, only approximate values can be given, as the methods for determination are not standardized. However, all these data fit the classification according to morphological characters.

Between groups 7 and 8, as well as between groups 10 and 11, there are only diminutive differences in NL, and it is rather difficult to prove them. Because of the great resemblance in regard to other characters it seems permissible to collect these groups in higher systematic units. One could think of a common origin of these viruses.

Nothing is known about the relationships among the other groups. Nevertheless it might not be fortuitous that viruses within the first three groups are not transmitted by aphids and that they have a high TIP. In addition viruses of groups 1 and 2 are soil borne.

Insect transmission occurs within groups 7–12 but the viruses are sap transmissible too. With the exception of beet yellows virus (group 12), all the viruses are nonpersistent. There are even serologically related viruses differing in the mode of transmission, e.g., potato virus Y and C (Watson, 1956). The same is true for potato viruses S and M and the carnation latent virus. The latter virus and some strains of virus M (Rozendaal and van Slogteren, 1958; Wetter and Völk, unpublished) are aphid transmissible, while the paracrinkle strain of virus M (Kassanis, 1956) and virus S are transmitted mechanically only. In general, however, the mode of transmission shows correlations within each group and the ability of a virus to be transmitted by insects or by particular types of insects probably has significance for the different morphological groups. There are reasons for supposing that failure of a virus to be transmitted by insects that transmit viruses belonging to the same group and related to it by other qualities is of derivative nature and without such significance.

In the twelve groups only such viruses are listed as are commonly taken for "species." A distant serological relationship between species is not excluded. Regarding TMV and cucumber green mottle virus, Knight (1955) convincingly showed that differences in chemical structure justify separation into two species in spite of some common antigenic groups. Differences of that order apparently are present between the bean form of the cowpea virus and type TMV (Bawden, 1958). It seems reasonable to use the term "genus" for such groups, as has been proposed for the potato viruses S and M and the carnation latent virus which are serologically related but nevertheless distinct species (Bagnall *et al.*, 1959).

While no differences in length could be detected between type TMV and cucumber green mottle virus, Bawden supposed the particles of the bean form to be shorter. Using the exudate method, comparative meas-

urements of particles of the type TMV and the bean form of the cowpea virus have revealed no distinct differences (Brandes, 1959).

Of course, because of the limited accuracy of the measuring procedure, the possibility that there may exist minor differences in length between serologically related viruses cannot be excluded.

This question is of great importance for classification, but it has not yet been answered.¹ In general, the morphology of the particles outweighs serological relationship.

There is only scarce and unreliable information on the shape and the size of the so-called spherical plant viruses, which in some cases have proved to be polyhedral. These viruses can possibly be classified according to their morphology in a similar way as elongated viruses. More recent results (Steere, 1958) indicate that the shape as well as the diameter can be used for differentiation.

Until now, morphology has been neglected in the description of plant viruses. As demonstrated above, NL and shape are suitable qualities for characterizing elongated viruses. The morphological data should be taken as a basis to start planned serological investigations on relationships among viruses.

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¹ Recent studies by Wetter and Quantz (1958) indicate a distant serological relationship between red clover vein mosaic virus (NL 650 m μ) and Wisconsin pea streak virus (NL 620 m μ), but this result needs confirmation by further investigations.

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