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Apparent Symbiotic Interaction Between Particles of Tobacco Rattle Virus

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SUMMARY

The predominant lengths of tubular particles produced by the CAM isolate of tobacco rattle virus (520 and 1950 Å long) and of the PRN isolate (650 to 800 and 1900 Å long) were separated by density gradient centrifugation. On their own, short particles of each isolate neither caused lesions in *Chenopodium amaranticolor* leaves nor multiplied detectably. Long particles of either isolate caused lesions which contained virus nucleic acid, but virus particles were not found in extracts of the lesions; when the lesions were thawed after 3 days storage at −15°, the extracts had little or no infectivity. When short particles were mixed with long particles of the same isolate, the number of lesions produced by the long particles was not affected, but a proportion of these lesions then contained tubular virus particles of both lengths, and also infective material resistant to freezing and thawing. This proportion increased from zero to unity with increasing concentration of short particles in the inoculum; it also increased with increasing susceptibility to infection of the plants used as the *source* of the lesions, but seemed unaffected by the concentration of long particles in the inoculum. With isolate CAM, the lesions containing stable virus particles could be distinguished by their smaller necrotic centres. When short particles were ultraviolet-irradiated, their ability to interact with long ones was abolished, but the interaction was not prevented by removing virus protein from both long and short particles by phenol treatment. No interaction was detected between particles of the two different isolates, which are distantly related serologically, or between their nucleic acids. Each isolate of tobacco rattle virus seems to be a system of two or more pieces of infective nucleic acid interacting specifically in a symbiotic manner.

INTRODUCTION

All unfractionated preparations of tobacco rattle virus contain tubular particles of more than one length. Paul & Bode (1955) found that two lengths, 700 Å and 1800 Å, predominated in samples of three German isolates, and similar figures have since been given for some other isolates but not all. Harrison & Woods (1966) found that ten isolates all had long particles about 1900 Å long, and all had one or more predominant length of shorter particles, which was characteristic of the isolate. The predominant short particles were about 450, 520, 790, 890, 1000 or 1120 Å long; some isolates produced only one of these kinds of short particles, others two or three.

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Isolates deemed closely related by other criteria did not necessarily have short particles of the same length. The ten isolates examined by Harrison & Woods (1966) fell into three major serological groups, one of which was possibly heterogeneous. Antigenic constitution bore little relation to the length of short particles produced by an isolate, but was related to the continent of origin of the isolate (Western Europe, North America or South America).

Pea early-browning virus also produces particles of two predominant lengths. Isolates from The Netherlands and from Britain each had lengths of about 1030 and 2120 Å (Bos & van der Want, 1962; Harrison, 1966), though a Dutch isolate was serologically only distantly related to the British ones (Gibbs & Harrison, 1964). Maat (1963) claimed that Dutch pea early-browning virus was serologically very distantly related to a Dutch isolate of tobacco rattle virus, but no relationship was detected between isolates of pea early-browning and tobacco rattle viruses from Britain (Gibbs & Harrison, 1964). A further difference is that although they have some hosts in common, tobacco rattle and pea early-browning viruses cause different diseases of economic importance and are readily distinguished in several indicator species.

Slow progress has been made in understanding the origin and functions of the particles of different characteristic lengths produced by any one isolate of these viruses. Fractionating preparations of the PRN isolate of tobacco rattle virus by two cycles of centrifugation in sucrose density gradients greatly purified the short particles (750 Å long), and freed the long particles (1850 Å long) from most of the short ones (the ratio of long to short particles in the unfractionated preparation was 1:5 and in the long-particle preparation was 8:1). When such preparations were used at the same number of particles per ml., those of long particles were about 5000 times more infective. The two kinds of particle seemed antigenically identical and both contained nucleic acid, but only the RNA from the long particles caused lesions in plants (Harrison & Nixon, 1959*a*, *b*). The short particles apparently did not influence the number of lesions produced by the long particles and seemed not to be infective. An isolate of pea early-browning virus from Britain gave similar results (Harrison, 1966).

A second novel feature of tobacco rattle virus is that some local lesions produced in tobacco leaves by dilute inocula yield very infective extracts, whereas others do not. When the lesions produced by the very infective extracts are further subcultured, again some yield highly infective extracts and others do not. By contrast, subculturing from the few lesions produced by the poorly infective extracts does not give extracts with increased infectivity (Cadman & Harrison, 1959). Sap from leaves systemically infected with isolates difficult to transmit was also only slightly infective, whereas extracts made with the aid of phenol were highly infective (Sänger & Brandenburg, 1961; Cadman, 1962). This difference suggested that the leaves contained much infective RNA but few or none of the stable nucleoprotein virus particles. However, the RNA seemed protected to some extent because, unlike the free virus nucleic acid produced by variants of tobacco necrosis virus (Babos & Kassanis, 1962; Kassanis & Welkie, 1963), its infectivity was not immediately lost when leaves were ground in water (Cadman, 1962).

Lister (1966) described results that could explain much of this intriguing behaviour. He found little infectivity in sap from 98 out of 100 lesions produced by inocula

containing predominantly long particles, obtained from the lower ultraviolet-absorbing zone when tobacco rattle virus was centrifuged in sucrose density gradients, whereas extracts of 67 out of 100 lesions produced by samples containing predominantly short particles from the top zone were highly infective. When he used mixtures of samples from the top and bottom zones as inocula, 'more than the expected proportion of infections' contained readily transmissible virus. Lister therefore suggested that the RNA of the long particles lacks information contained in the short ones that is needed at some stage before virus protein assembles on the RNA. Poorly transmissible isolates lack this information.

The experiments described below were made to test Lister's hypothesis and to gain more information on the nature of the interaction between the two kinds of particle. An isolate of tobacco rattle virus from Brazil was used in most experiments because its short particles are only 520 Å long and therefore more readily separated from the long particles than those of most other isolates, and because it is only distantly related serologically to the isolates from Western Europe used by Lister (1966).

METHODS

Viruses. The two isolates of tobacco rattle virus used, CAM (from Brazil) and PRN (from Scotland), could be distinguished serologically, by the lengths of their short particles, and by their effects on plants (Harrison & Woods, 1966). Both isolates were propagated in *Nicotiana tabacum* L. cv. White Burley in a glasshouse at about 20°. Inoculated and systemically infected leaves containing isolate CAM were harvested after 14 days; leaves of plants inoculated with isolate PRN were harvested after 4 to 6 days. The infected leaves were minced, and the sap pressed out through muslin and stored at $-15°$ for about a month.

Virus purification. About 150 ml. of frozen sap were thawed, mixed with an equal volume of 0·07 M-phosphate buffer (pH 7·8), and centrifuged for 3 min. at 8000*g*. The virus was obtained from the supernatant fluid by two cycles of differential centrifugation, and the pellets from the high speed centrifugations were resuspended in 0·02 M-phosphate buffer (pH 7·8). The final preparations (about 4 ml.) contained 2 to 4 mg. virus per ml. and some showed anisotropy of flow when rocked in plane-polarized light.

Virus fractionation. Long and short particles of each isolate were separated by centrifuging in sucrose density gradients. These were made by layering 4, 7, 7 and 9 ml., respectively, of solutions containing 100, 200, 300 and 400 g. sucrose per litre of 0·02 M-buffer, and were kept overnight at 5° before use. Either 1·0 or 1·5 ml. of the virus preparation was floated on each of three density gradients, which were then centrifuged in a M.S.E. 3×40 ml. swinging-bucket rotor at 21,000 rev./min., at 5° for 1·5 hr. The fractions were obtained from the gradients by puncturing the bottom of each tube, pumping in sucrose solution (600 g./l.) at about 1 ml. per min., passing the liquid that was displaced from the top of the tube through a short length of 1·2 mm. bore Teflon tubing attached to a LKB Uvicord flow absorptiometer, and collecting the samples at the end of a second short piece of Teflon tubing attached to the outlet of the flow cell of the absorptiometer, which was coupled to a continuously reading chart recorder. The whole apparatus was operated at 5°. Fractions

corresponding to the middle parts of the ultraviolet-absorbing peaks on the chart were collected, allowing for the volume lag between passage through the cell and collection (Fig. 1, top). After the zone containing the short particles had passed through the flow cell, the pump was stopped and the flow cell and connecting tubes flushed several times with water to remove remaining short particles, which might otherwise contaminate the fraction containing the long particles. The individual long- and short-particle fractions were pooled, respectively, and dialysed overnight against cold 0·02 M-buffer to remove sucrose. The two samples were then concentrated, in readiness for the second density gradient centrifugation, by suspending the dialysis sacs in polyethylene glycol (Carbowax, 20M; G. T. Gurr, Ltd.).

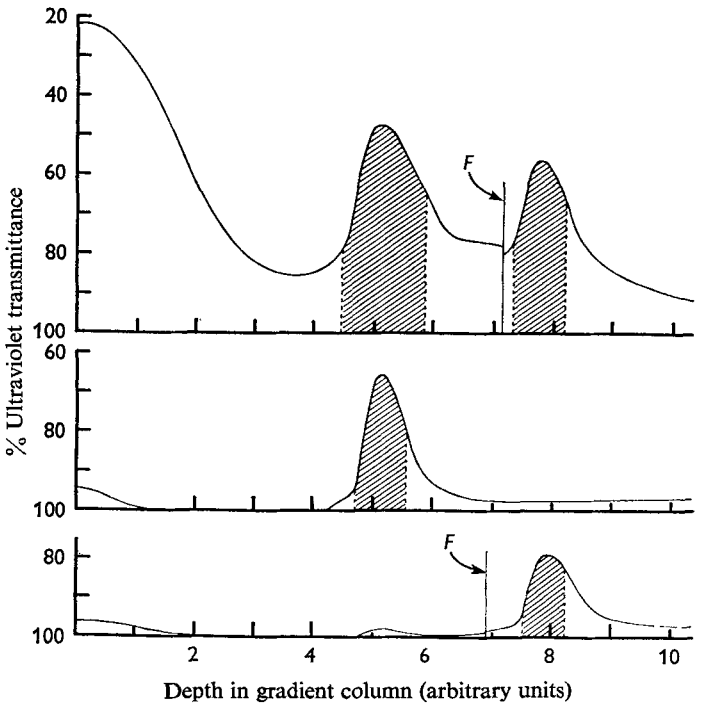

Fig. 1. Fractionation of ultraviolet-absorbing material of isolated CAM in sucrose density-gradient columns. The cross-hatched areas represent the fractions collected. The vertical line at *F* shows when the pump was stopped and the flow cell flushed out. For full description, see text. Top curve: analysis of purified virus preparation; much of the ultraviolet absorption near the meniscus probably was caused by host plant material. Middle curve: analysis of short-particle fraction obtained after one density gradient fractionation. Bottom curve: analysis of long-particle fraction obtained after one density gradient fractionation.

Virus fractions (1 ml.) were floated on each of the second set of density gradients, which were centrifuged and fractionated as before (Fig. 1, middle and bottom). The individual long- and short-particle fractions were again pooled, and dialysed 3 days against 0·02 M-buffer. These preparations contained about 5 % and 15 % respectively of the long and short particles in the crude sap.

Estimation of particle concentrations. A modification of the spray-droplet method of Nixon & Fisher (1958) was used. Virus preparation (0·2 ml.) was mixed with

0·15 ml. of 1 % (w/v) neutral sodium phosphotungstate, 0·05 ml. of 0·1 % (w/v) bovine serum albumin and 0·2 ml. of a standard suspension of 880 Å diameter polystyrene latex particles (Dow Chemical Co.). This mixture was sprayed with an airbrush into a Cascade Impactor, and droplets giving traces about 3 to 5 μ diameter were collected on a pyroxylin-covered grid and examined at $\times$20,000 magnification in a Siemens Elmiskop I electron microscope. Enough virus particles were counted to give a maximum standard error of the ratio of virus to latex particles of 15 %.

Particle-length measurements. These were made as described by Harrison, Nixon & Woods (1965).

Preparation of virus nucleic acid. Nucleic acid was extracted from purified virus preparations by treatment with phenol as described by Harrison & Nixon (1959*b*).

Assay of infectivity. Virus preparations were manually inoculated on the four youngest expanded leaves of *Chenopodium amaranticolor* Coste & Reyn. Carborundum powder (600-mesh) was dusted on the leaves before inoculation to increase the sensitivity of the assay, and the local lesions were counted about 7 days after inoculation.

When virus was cultured from single, well-separated lesions, these were cut out with a cork borer (6 mm. diameter) about a week after inoculation. The lesions were then stored for 2 days or more at $-15°$ before being thawed and ground with a single drop of water to give an inoculum. In this way the infectivity of nucleoprotein particles was preserved but the unstable infective material produced by poorly transmissible isolates was mostly inactivated (Lister, 1966). Each inoculum was applied to two *Chenopodium amaranticolor* leaves.

Ultraviolet irradiation. A shallow layer of the appropriately diluted short-particle fraction was irradiated for 4 min. at 1500 μW/cm^2, using a Hanovia low-pressure mercury-vapour lamp with its output almost exclusively at 2537 Å.

Table 1. *The proportions and concentrations of tobacco rattle virus (CAM isolate) particles of different lengths at stages during purification and fractionation*

Type of preparation	No. particles of different lengths (10^{10}/ml.: percentages in parentheses)		Volume of preparation (ml.)
	< 900 Å	> 900 Å	
Thawed sap	230 (84)	44 (16)	160
Purified virus	7280 (83)	1470 (17)	4·5
Short-particle 1st gradient	2250 (99·9)	*c.* 2* (0·1)	3·5
2nd gradient	672 (100)	*c.* 0·01*	8
Long-particle 1st gradient	119 (26)	339 (74)	3·5
2nd gradient	6 (7)	77 (93)	3·5

* Estimated from electron microscopy of an undiluted preparation, and from infectivity tests.

RESULTS

Fractionation, and properties of the fractions

Table 1 shows the ratios and numbers of particles at different stages during preparation of the long- and short-particle fractions of isolate CAM. The two categories, < 900 Å and > 900 Å, conveniently separate the two predominant lengths characteristic of isolate CAM, about 520 and 1950 Å long respectively (Harrison & Woods,

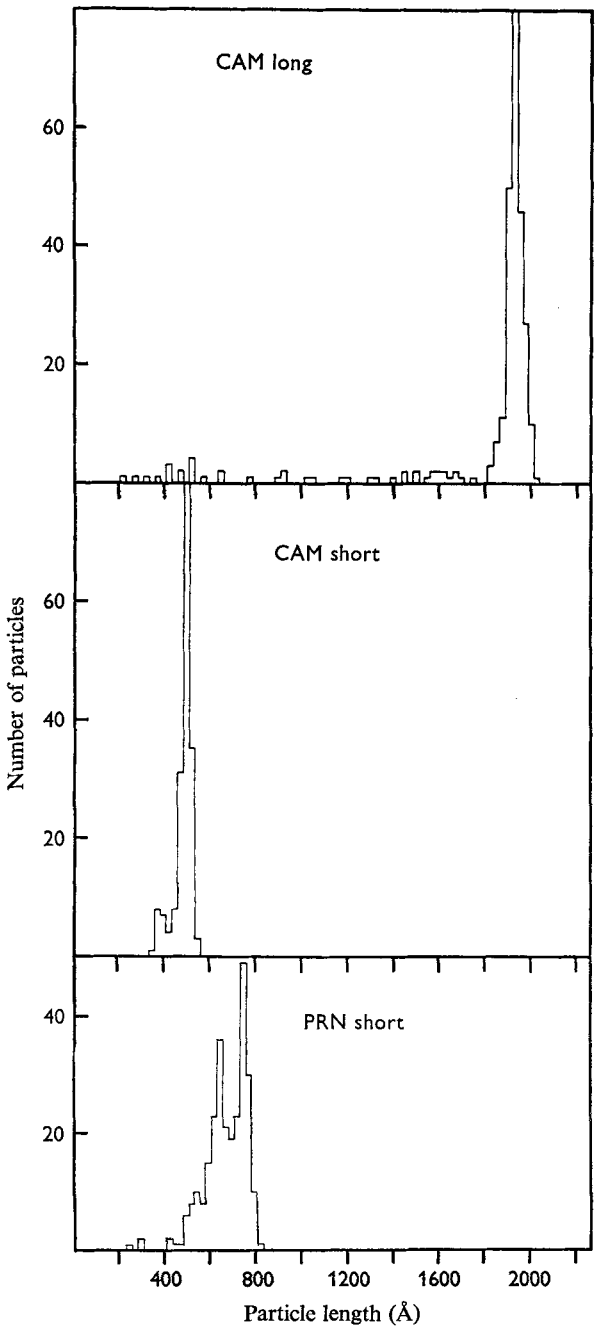

Fig. 2. Distribution of lengths of particles in preparations of tobacco rattle virus after two density-gradient fractionations. Top histogram, long-particle fraction of isolate CAM (278 particles); middle, short-particle fraction of isolate CAM (177 particles); bottom, short-particle fraction of isolate PRN (266 particles).

1966). Preparations of short particles containing few long ones were obtained without great difficulty but preparations of long particles all contained shorter ones, possibly because a few long particles break, because some aggregates that sediment at a similar rate to long particles disrupt, or because of 'non-ideal' sedimentation (Brakke, 1964). Similar results were obtained with isolate PRN, though because the short particles of this isolate are longer than those of isolate CAM, the long and short particles were less well separated.

To interpret the behaviour of the fractions and their mixtures, their composition should be known accurately. Figure 2 shows the distributions of particle lengths in gross-drop mounts of the final long- and short-particle fractions. The concentration of particles in the PRN long fraction was too small for enough to be photographed and measured. These diagrams again show that whereas short particles can readily be freed from long ones the reverse is more difficult. They also show that the short particles of isolate CAM were more uniform in length than those of isolate PRN.

In the mounts where they were the most numerous, CAM short particles seemed to pack side to side in a special way. This could be seen both when the particles were viewed side-on, and also when they stood on end in thicker films of phosphotungstate (Plate, figs. 1, 2). The particles on end were sometimes tilted as though they were parts of a helically twisted strand seen in cross-section, possibly because of surface-tension forces applied during drying (Plate, fig. 2). Of more than 10^4 particles examined in this preparation of CAM, all were short. The electron micrograph of the CAM long-particle fraction, however, shows a few short particles (Plate, fig. 3); it also shows the tendency of the long particles to aggregate side to side.

When inoculated at a concentration of 10^8 long particles per ml., the long-particle fraction of isolate CAM usually caused about 15 lesions per *Chenopodium amaranticolor* leaf. The short-particle fraction of isolate CAM, when inoculated at a concentration of 5×10^{10} particles per ml., caused 0 to 0·3 lesions per leaf in different experiments, suggesting that it contained 1 long particle in 5×10^4 short ones. At a concentration of 10^8 long particles per ml., the long-particle fraction of isolate PRN caused about 4 lesions per *C. amaranticolor* leaf, from which it was calculated that the short-particle fraction contained about 1 long in 5×10^3 short particles.

Infection by fractions and artificial mixtures

When increasing numbers of CAM short particles were added to constant numbers of CAM long particles there was no consistent effect on the numbers of lesions produced in *Chenopodium amaranticolor* leaves (Table 2). However, the sort of lesion that developed was affected, for lesions caused by long-particle fractions alone had larger necrotic centres than most of those caused by inocula also containing many short particles. The relative proportions of the two types of lesions depended on the concentration of short particles in the inoculum (Table 3) and seemed unaffected by varying the concentration of long particles. However, the two kinds of lesions differed only slightly in appearance and it was sometimes difficult to allocate every lesion on a leaf to one category or the other.

When virus was cultured from single lesions produced by inocula containing a standard concentration of CAM long particles and different concentrations of short ones, great differences were found. Whereas none of the extracts from single lesions

produced by the long-particle fraction alone gave as much as one lesion per leaf, extracts from many of those produced by inocula containing both long- and short-particle fractions gave 100 or more lesions per leaf. Although this phenomenon was reproduced consistently, the actual numbers of lesions given by the two kinds of single lesions differed in different experiments. For instance, in the experiment quoted in Table 3, the lesions caused by the long-particle fraction gave an average of 0·01 lesions per leaf when subcultured, and those initiated by the mixture containing the most short particles yielded 98 lesions per leaf; in a second experiment using the same two inocula the figures were 2, and more than 1500. The actual numbers obtained in different experiments seemed to depend mainly on the relative susceptibility to infection of the assay plants. Lesions from which virus was readily cultured are referred to below as V-type lesions and those from which virus was cultured with difficulty, or not at all, are called N-type lesions.

Table 2. *Effect on lesion number of different concentrations of long and short particles of isolate CAM*

Content of inoculum*				
Short particles/ml.	Long particles/ml.			
	2×10^{9}	2×10^{8}	2×10^{7}	0†
0†	1049‡	118	11	—
2×10^{9}	1070	174	25	0
10^{10}	1487	124	11	0
5×10^{10}	1428	137	22	4
5×10^{10} (u.v.-irradiated)	1170	277	28	—

* All dilutions were made in 0·02M-phosphate buffer (pH 7·8).
† None added to short-particle and long-particle fractions, respectively.
‡ Figures are total numbers of lesions on 12 leaves (3 plants) of *Chenopodium amaranticolor*.

Table 3. *Effect of short particles on the form of, and content of stable virus in, lesions caused by long particles of isolate CAM*

Content of inoculum (10^{8} particles/ml.)		No. lesions with small necrotic centre/total no. examined (percentages in parentheses)	No. V-type lesions/ total no. tested* (percentages in parentheses)	Mean no. lesions/ leaf produced by single-lesion isolates
Long particles	Short particles			
2	0†	0/85 (0)	0/46 (0)	0·01
2	20	21/214 (10)	5/50 (10)	8
2	100	148/317 (46)	14/38 (37)	10
2	500	263/319 (83)	24/29 (83)	98
2	500 (u.v.-irradiated)	6/223 (3)	0/50 (0)	0·00

* V-type lesions gave 3–260 lesions/leaf, N-type lesions gave < 1 lesion/leaf.
† No short particles were added to the long-particle fraction.

The proportion of V-type lesions depended on the concentration of short particles in the inoculum, and was closely parallel to the proportion of lesions with small necrotic centres (Table 3). About 10^{11} short particles per ml. ensured that all lesions were V-type with small necrotic centres. The ability of short particles to affect lesion type in both these ways was abolished by ultraviolet irradiation (Table 3).

When crude sap from *Chenopodium amaranticolor* leaves with many lesions was examined in the electron microscope, virus particles were not seen in sap from leaves inoculated with the CAM long-particle fraction alone, but long and short tubular virus particles were plentiful in sap from leaves inoculated with artificial mixtures of CAM long and short particles. One virus-like particle was found in sap from leaves inoculated with long particles plus ultraviolet-irradiated short ones. The PRN isolate behaved much like CAM, except that V- and N-type lesions could not be distinguished by eye.

To see whether infective RNA was readily detectable in N-type lesions, extracts were made in three ways from leaves each bearing about 140 lesions produced by the long-particle fraction of isolate CAM. The leaves were picked and either (*a*) ground fresh at room temperature in nine times their weight of 0·02 M-phosphate buffer, or (*b*) were stored 2 days at $-15°$ before grinding, or (*c*) were ground fresh at 4° with buffer plus an equal volume of water-saturated phenol and a nucleic acid preparation obtained from the emulsion in the usual way. The extracts made with phenol caused at least 20 times as many lesions as those made with buffer alone, from either fresh or frozen leaves. Thus although the N-type lesions contained little or no stable infective material they did contain considerable amounts of infective nucleic acid.

Our results provide quantitative support for the hypothesis proposed by Lister (1966). Infections initiated by long particles alone result in lesions containing only unstable infective material, whereas those initiated jointly by long and short particles cause lesions containing stable nucleoprotein particles. The short particles seem to provide some otherwise lacking information that is needed for the synthesis of the coat protein of the virus or for its assembly with the nucleic acid.

Specificity of the interactions between particles

The nucleic acid of the short tobacco rattle virus particles may perhaps carry the 'code' that determines the sequence of amino acid residues in the viral coat protein. To test this possibility, the CAM long-particle fraction was mixed with the serologically distinguishable PRN short-particle fraction, and PRN long particles were mixed with CAM short ones. The two mixtures produced only 2 V-type lesions out of 80 tested (Table 4); these two probably resulted from the few contaminating long particles in the short-particle fractions. The few long and short particles found in saps from leaves inoculated with these mixtures are also most plausibly explained by these slight contaminations. By contrast, many V-type lesions were given by the two control mixtures of particles of the same isolate (CAM long + CAM short; PRN long + PRN short). It is interesting to note that PRN short particles were less effective than those of CAM in producing V-type lesions. This may reflect the fact that the PRN short particles were less uniform in length (Fig. 2), and perhaps fewer of them carried all the information needed for the interaction with long particles of the same isolate.

Interactions of extracted nucleic acids

The effect of ultraviolet-irradiation on the behaviour of CAM short particles suggested that the nucleic acid of the short particles is involved in their interaction with long ones, for the dose of ultraviolet-radiation used was too small to have much effect on virus protein. To see whether the coat protein of the virus was also needed,

the behaviour of mixtures of the nucleic acids extracted from the long- and short-particle fractions was tested. Nucleic acid prepared from long particles had 5 to 10 % of the infectivity of the parent preparation of nucleoprotein particles, but the proportion of the nucleic acid that was extracted from the particles was not determined.

The extracted nucleic acids of isolate CAM interacted in the same way as intact nucleoprotein particles (Table 5), showing that coat protein of the virus was not essential for the interaction. Also, sap from leaves inoculated with the mixed nucleic acids contained many long and short virus particles. Further tests showed that nucleic acid from the CAM short-particle fraction did not interact with nucleic acid of the PRN long-particle fraction to produce nucleoprotein particles. Hence the failure of the two isolates to interact could not depend merely on failure of the nucleoprotein particles to uncoat during infection.

Table 4. *Form of, and content of stable virus in, lesions caused by mixtures of long and short particles of different virus isolates*

Content of inoculum (10^7 particles/ml.)		No. of lesions with small necrotic centres/total no. examined (percentages in parentheses)	No. V-type lesions/total no. tested* (percentages in parentheses)
Long particles	Short particles		
3·4 CAM	0†	0/200 (0)	0/40 (0)
3·4 CAM	5000 CAM	267/294 (91)	39/40 (98)
3·4 CAM	5000 PRN	3/165 (2)	1/40 (3)
6·1 PRN	0†	—	0/20 (0)
6·1 PRN	5000 CAM	—	1/40 (3)
6·1 PRN	5000 PRN	—	8/20 (40)

* V-type lesions gave 5–340 lesions/leaf, N-type lesions gave < 2 lesions/leaf.
† No short particles were added to the long-particle fraction.

Table 5. *Interaction between nucleic acids, and between nucleoprotein particles, of isolate CAM*

Content of inoculum (10^7 particles/ml.)		No. V-type lesions/total no. tested* (percentages in parentheses)
Long particles	Short particles	
6·9	0†	0/20 (0)
6·9	5,000	20/20 (100)
34 (NA)‡	165,000 (NA)‡	30/30 (100)

* V-type lesions gave 120–2,000 lesions/leaf, N-type lesions gave < 15 lesions/leaf.
† No short particles were added to the long-particle fraction.
‡ Number of particle-equivalents of viral nucleic acid, assuming complete extraction of nucleic acid from the nucleoprotein particles.

DISCUSSION

The interaction between long and short particles of tobacco rattle virus seems different from any phenomenon reported with other plant viruses. The relation between tobacco necrosis virus and its satellite virus (Kassanis & Nixon, 1961; Kassanis, 1962) differs from the tobacco rattle virus system because tobacco necrosis virus multiplies to form nucleoprotein particles on its own, and the coat proteins of the interacting

viruses are unrelated serologically; it is apparently of advantage only to the satellite virus and can be likened to parasitism. By contrast, the interaction of long and short tobacco rattle virus particles is advantageous to each; it seems analogous to symbiosis. The short particles apparently do not multiply on their own, and the long particles, although able to cause lesions in plants and to replicate their nucleic acid, apparently cannot produce the stable nucleoprotein particles. The interactions between the particles of *Prunus* necrotic ringspot virus (Fulton, 1962) and between the 91 S and 112 S components of either bean pod mottle or cowpea yellow mosaic viruses (Wood & Bancroft, 1965) differ from the tobacco rattle virus system because they enhance the number of lesions produced, whereas short tobacco rattle virus particles do not affect the number of lesions caused by long ones.

Long and short tobacco rattle virus nucleoprotein particles were produced only when both, or their nucleic acids, were in the inoculum. N- or V-type lesions can therefore be obtained at will by using inocula containing long particles only, or long plus short particles. That diluted sap often produces mainly N-type lesions (Cadman & Harrison, 1959) is readily explained by the small chance of long and short particles infecting the same site. Whether short particles on their own induce the replication of their nucleic acid without causing symptoms still requires testing by experiment. However, the need to have a great excess of short particles to ensure that some interact with the many fewer long ones suggests that the nucleic acid of the short particles does not replicate on its own.

As a working hypothesis, it is suggested that long and short particles infect sites independently, and that the chance they will both infect the same site depends on their concentration in the inoculum and the volume of inoculum to which the site is exposed. It is interesting to compare the frequency with which the particles interacted in leaves differing in susceptibility to infection with the long particles. Mixtures containing long and short particles, with the short particles at a constant concentration, were inoculated on different days to three batches of plants. Five times more long particles were needed to cause 10 to 12 lesions per leaf on the first batch than on the second and third batches. The percentages of V-type lesions were 83, 98 and 100, respectively; that is, larger for the more susceptible *source* plants. Plants may thus be more susceptible to infection at least in part because their infectible sites are, on average, larger or because for some other reason larger amounts of inoculum reach them.

The amounts of infective material detected in different single V-type lesions obviously will have depended on the susceptibility to infection of the *assay* plants. However, for any one group of assay plants these amounts seemed more uniform when the source lesions were mostly V-type than when only few were. This could be explained if more than one short particle can take part in the interaction at an infectible site, and the effects of additional short particles are to some extent cumulative. It could also be explained by short particles persisting, in cells adjacent to those infected by long particles, until these cells are invaded by the nucleic acid of long particles. The interaction would then be delayed and might occur only in one part of a lesion.

Lister (1966) referred to observations suggesting the interaction of particles of different, serologically closely related strains of tobacco rattle virus, but not those

of tobacco rattle virus and a British isolate of pea early-browning virus. We found that particles of isolates belonging to different serotypes of tobacco rattle virus did not interact, so ability to interact may be specific to particles of closely related strains. Our results show that the inability of particles of the different serotypes to interact does not depend solely on a possible specificity of the hypothetical mechanism of uncoating the infecting particles. Nor does it depend solely on a specific coating mechanism when new virus particles are assembled, because if coating were specific then either long or short particles should accumulate in large numbers in leaves inoculated with PRN long plus CAM short particles, and they do not. The simplest explanation is that the nucleic acid of the short particles cannot replicate without aid of a specific kind (e.g. a specific RNA polymerase) and that this is specified only by the nucleic acid of the homologous long particles.

Although we have emphasized the differences between the tobacco rattle virus system and the tobacco necrosis virus/satellite virus system, there are also similarities. In both the interactions can be reproduced by inocula free from virus protein, in both the necrotic area of a lesion is smaller when the interaction occurs than when it does not, in both the smaller particle does not replicate on its own and in both the two kinds of infecting particle seem to reach infectible sites independently. It would not be surprising to find further similarities in the mechanisms of interaction.

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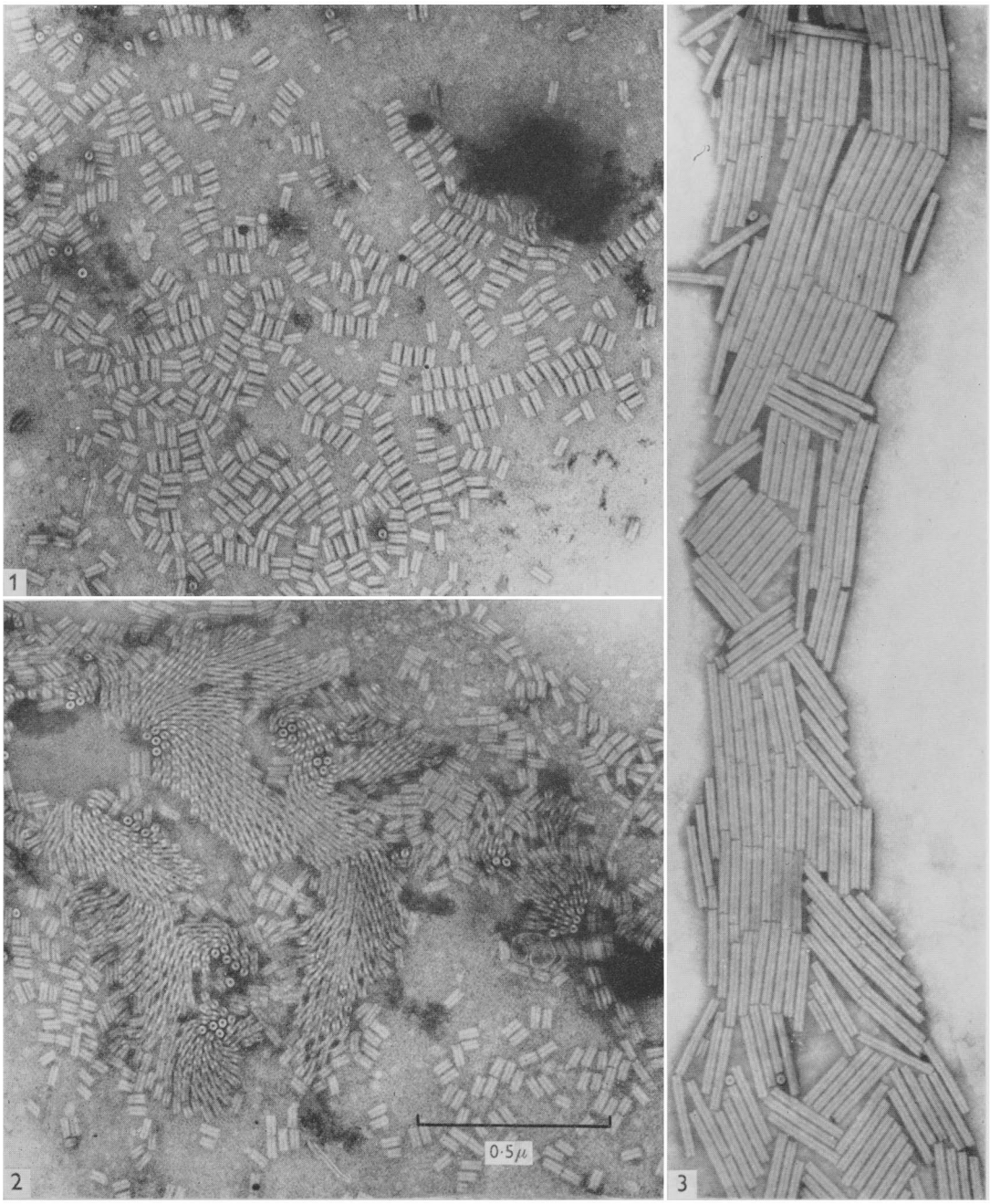
EXPLANATION OF PLATE

Figs. 1–3 are all at the same magnification.

Fig. 1. Electron micrograph of gross-drop mount of the short-particle fraction of isolate CAM, showing particles lying side-by-side in short rows. Mounted in sodium phosphotungstate.

Fig. 2. As fig. 1, but many particles viewed almost end-on in aggregates.

Fig. 3. Electron micrograph of gross-drop mount of the long-particle fraction of isolate CAM.



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(*Facing p.* 70)