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# Comparative Effects of Temperature on the Multiplication in Tobacco Leaves of Two Tobacco Rattle Viruses

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SUMMARY

Two kinds of assay, particle counts by electron microscopy and infectivity for leaves of *Phaseolus vulgaris* were used to estimate the amounts of virus in extracts made from tobacco leaves kept at 14 to 34° for 1 to 6 days after inoculation, and in extracts from uninoculated tip leaves sampled 14 days after the plants were inoculated. One, 2 and 6 days after inoculation, virus isolate PRN was obtained in largest amount from leaves at 22°, 18° and 14°, respectively. Isolate CAM showed the same trend, but equivalent temperatures were 4 to 6° higher. At 14° infectivity and particle numbers continued to increase between 1 and 6 days after inoculation but at 30° infectivity decreased after 1 day. At some temperatures the number of particles first increased and then decreased. For instance, at 26° the number of particles of isolate CAM tripled between 1 and 2 days, and then decreased at least 100-fold between 2 and 6 days after inoculation. Temperature had only small effects on the ratio of long (*c.* 1900 Å) to short (mainly 450 to 900 Å) virus particles. Specific infectivity increased slightly when particle number was increasing and decreased greatly when particle number was decreasing. Specific infectivity sometimes decreased slightly before particle number. As with some small isometric plant viruses, the amount of tobacco rattle virus extracted from leaves apparently represents the resultant between synthesis and degradation, and the two processes are differently affected by temperature. At 14 to 22° but not at 26°, isolate PRN produced necrotic lesions in inoculated leaves, whereas isolate CAM did not cause visible lesions. This difference seemed to have little effect on the changes in virus titre but although isolate PRN multiplied optimally at a lower temperature than CAM, it was more stable *in vivo* at 26°. Isolate PRN invaded uninoculated leaves only sporadically and accumulated in only small amounts. Isolate CAM readily invaded uninoculated leaves, in which it reached its greatest concentration at 22°.

## INTRODUCTION

Recent work has shown some of the complexities in the multiplication of tobacco rattle viruses (Harrison & Woods, 1966; Lister, 1966; Frost, Harrison & Woods, 1967). The long and short tubular nucleoprotein particles characteristic of any one virus isolate seem to interact specifically in a manner analogous to symbiosis. The long and short nucleoprotein particles are produced only by inocula containing both kinds of particle or the RNA from both kinds. The long particles can infect plants and cause lesions in inoculated leaves but, although extracts made from these lesions with phenol are very

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infective, extracts made with buffer alone do not contain tubular nucleoprotein particles and have little or no infectivity. The short particles do not cause lesions in plants and seem to fail to multiply; extracts from inoculated leaves do not contain tubular particles, but the possibility that the RNA of the short particles replicates without causing symptoms cannot be completely excluded. Lister (1966) suggested that the RNA in the long virus particles lacks information needed at some stage in the process leading to assembly of the virus coat protein with virus RNA, whereas the RNA in the short particles, though containing the information lacking in the long particles, cannot mediate one or more of the other stages in the multiplication process. The results of Frost *et al.* (1967) support this.

How temperature affects the complementation between long and short particles is unknown. The experiments we describe were made to study the effect of temperature during the whole process of virus infection and multiplication, and to see whether the results would give some insight into the nature of the complementation. We used two contrasting tobacco rattle viruses, originally obtained from different continents. They are serologically only distantly related, have short particles of different predominant lengths and cause different symptoms in tobacco plants. The effects of temperature were determined by assaying the infectivity of leaf extracts and their total content of virus particles. In this way the accumulation of long and short particles, the proportion of each, and the specific infectivity of the long particles could be studied. Results are given for only one experiment at each temperature but duplicate experiments yielded similar figures.

## METHODS

*Viruses.* The two isolates used, CAM and PRN, were those also used by Frost *et al.* (1967) and were described by Harrison & Woods (1966). Isolate CAM was originally obtained from *Bidens* sp. in Brazil and was kindly supplied by Dr A. S. Costa, Campinas, Brazil. It infected tobacco (*Nicotiana tabacum* L. cv. White Burley) systemically and rarely produced lesions in the inoculated leaves; fine necrotic patterns formed in the systemically infected leaves of some plants. Its predominant particle lengths were 520 and 1970 Å. Isolate PRN was originally obtained from *Solanum tuberosum* L. in Scotland (Cadman & Harrison, 1959). It produced necrotic local lesions in inoculated tobacco leaves, and in some plants caused localized necrosis of uninoculated tip leaves. Its predominant particle lengths were 780 and 1910 Å. To prepare the standard inocula for the experiments, tobacco leaves systemically infected with isolate CAM were harvested 14 days after the plants were inoculated; leaves inoculated with isolate PRN were picked after 5 days. The leaves were minced, the sap pressed out through muslin, and 2·5 ml. portions stored at $-15°$.

*Experimental plants.* White Burley tobacco plants, raised at about 22° were used when about 10 weeks old. Their two youngest fully expanded (7 to 12 cm. long) leaves were dusted with 600-mesh carborundum powder and rubbed with the standard inocula, diluted with distilled water to contain a constant number (approximately $10^{12}$) of particles per ml. The inoculated leaves were carefully rinsed in a stream of tap water to remove excess inoculum, and the plants immediately placed in miniature glasshouses (Kassanis, 1954) in which the temperature could be controlled to $\pm 1°$. There were sixteen replicate plants per temperature treatment and each experiment included either three or four treatments, of which one was always 22°.

The inoculated tobacco leaves were sampled after 1, 2, 3 and 6 days at each tempera-

ture. The uninoculated leaf next in age to the inoculated ones was sampled after 14 days to test for systemic infection. At each sampling, 64 discs (13 mm. diameter) were cut with a cork borer, equal numbers from the tips, middles and bases of leaves of each age. The leaf discs were immediately ground with a pestle in a small wet mortar containing a little 100-mesh carborundum. The pulp was washed from the pestle and mortar with three drops of water and centrifuged for 3 min. at 8000 ***g***. The supernatant fluid was then stored at $-15°$ until virus assay.

*Infectivity assay.* The infectivity of the five samples (1, 2, 3, 6 and 14 days) from each temperature was compared with the infectivity of a standard inoculum, using the primary leaves of beans (*Phaseolus vulgaris* L. cv. The Prince). Immediately before inoculation a sample of the standard inoculum and the experimental samples were thawed and then centrifuged for 3 min. at 8000 ***g***. The standard inoculum was diluted 1/125, to give about 100 lesions per half-leaf. Each experimental sample was prepared at three serial fivefold dilutions, where possible chosen (after preliminary tests) to give about 250, 50 and 10 lesions per half-leaf. Each dilution of each of the five experimental samples was inoculated to ten half-leaves (dusted with carborundum) for comparison with the diluted standard inoculum; the inocula in batches of six were systematically distributed among the half-leaves of the plants. Two days (isolate PRN) or 3 days (isolate CAM) after inoculation the leaves were picked, decolorized by boiling in 70% ethanol for 5 min. and preserved in 70% ethanol until the lesions were counted at $\times 6$ magnification under a stereoscopic microscope.

The count of virus particles (see below) in the standard inocula decreased to about one-third during the 9 months in which the experiments and infectivity assays were made. The ratio of long to short particles did not change and there was no consistent change in the number of particles needed to give a lesion, so the infectivity assays of the experimental samples were adjusted to allow for the decrease in apparent virus content of the standard inoculum. The reciprocal of the dilution of each experimental sample that would have given the same number of lesions as the adjusted number given by the standard inoculum at 1/125 was found graphically, and used as the estimate of relative infectivity of the sample.

*Particle counts.* Particles were counted by the spray-droplet method, modified as described by Frost *et al.* (1967). Long and short particles were recorded separately; long particles of isolate CAM were arbitrarily designated as longer than 900 Å, and of isolate PRN as longer than 1350 Å. In all samples except those containing less than $5 \times 10^{10}$ particles/ml. at least fifty long particles were counted, giving a standard error of the ratio of long particles to polystyrene latex spheres of less than 15%.

## RESULTS

### *Effects on concentration of virus particles*

The smallest concentration of virus detectable by electron microscopy was about $3 \times 10^8$ particles/ml.; about $5 \times 10^{10}$ particles/ml. were needed to give a reliable ratio of long to short particles. Concentrations between $3 \times 10^8$ and $3 \times 10^9$ particles/ml. were recorded as trace amounts. In leaves inoculated with isolate CAM (Fig. 1A) virus particles were detected 1 day after inoculation at all temperatures except 14° and 34°. At 34° they were not detected at any time, but at 14° they were found after 2 days and steadily increased in number up to 6 days. At 18 to 22° the early part of the increase

was faster and the later part slower than at 14°. However, the most notable changes in particle concentration occurred at 26 to 30°. Peak concentrations of about $10^{12}$ particles/ml. were reached 2 days after inoculation, but particles then became fewer and none was detected 6 days after inoculation.

Isolate PRN (Fig. 1B) behaved similarly to isolate CAM, except that its temperature optima were 4 to 6° lower, and particles were never detected at 30°. Particles were found 1 day after inoculation in leaves at 14 to 26° but far the largest numbers were found at 18 to 22°. Between 2 and 6 days after inoculation, particle number increased steadily at 14°, decreased slightly at 18 to 22°, and at 26° first increased and then decreased. The decrease at 26° was, however, less than with isolate CAM, despite PRN seeming less able than CAM to multiply at 26°.

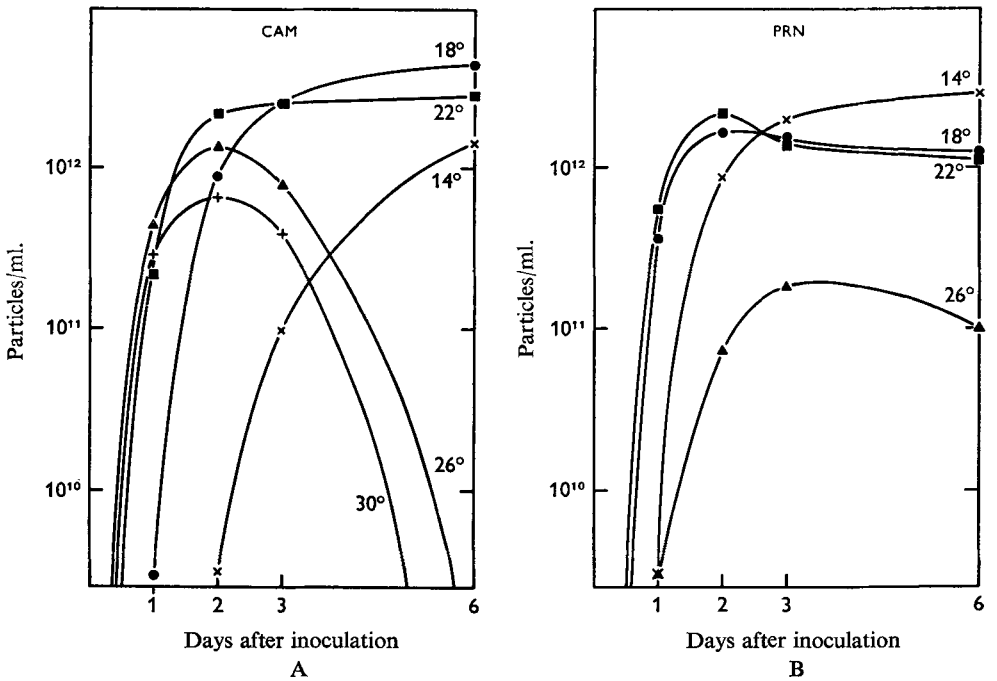


Fig. 1. Concentration of particles of tobacco rattle viruses in extracts made from inoculated tobacco leaves. A, Isolate CAM; B, isolate PRN.

The concentration of virus particles at any one temperature seems to reflect he resultant between the amount of virus synthesized and the amount broken down, with the rates of production and degradation being differently affected by temperature (Kassanis, 1954; Harrison, 1956). With isolate PRN, interpretation is complicated by the fact that necrotic lesions formed at 14 to 22°, though not at or above 26°. At 14° they appeared in about 3 days, at 18° in about 2 days and at 22° about 1 day after inoculation. It is perhaps not surprising that particle numbers increased only little after the lesions formed, because considerable areas of leaf were killed; but there was no good evidence that extraction of virus from leaves bearing the lesions was appreciably less efficient than from leaves without lesions. The trends established for isolate CAM, which did not cause necrotic lesions, in general held also

for isolate PRN. With no evidence to the contrary, we have assumed that a constant proportion of virus was extracted from the tissues.

The numbers of infections per inoculated tobacco leaf were affected by temperature; for instance, isolate PRN produced more at 14° than at 22°. How these differences affected virus accumulation is not clear, but with tobacco necrosis virus in *Phaseolus*

Table 1. *The concentration of 'long' virus particles in leaf extracts**

| Days after inoculation | No of particles/ml. ($\times 10^{10}$) at: | | | | | |
|---|---|---|---|---|---|---|
| | 14° | 18° | 22° | 26° | 30° | 34° |
| | | | (*a*) Isolate PRN | | | |
| 1 | T† | 10 | 18 | T | ND‡ | ND |
| 2 | 27 | 39 | 59 | 1·7 | ND | ND |
| 3 | 48 | 42 | 32 | 4·2 | ND | — |
| 6 | 34 | 29 | 24 | 1·8 | ND | — |
| 14§ | T | 0·46 | T | T | ND | — |
| | | | (*b*) Isolate CAM | | | |
| 1 | ND | T | 6·8 | 7·8 | 5·3 | ND |
| 2 | T | 35 | 79 | 29 | 11 | ND |
| 3 | 5·1 | 101 | 98 | 17 | 6·5 | ND |
| 6 | 52 | 131 | 107 | ND | ND | ND |
| 14§ | 4·2 | 76 | 86 | 11 | T | ND |

* 'Long' particles of isolate PRN were those > 1350 Å, and of isolate CAM were > 900 Å in length.

† T = $3 \times 10^8$ to $3 \times 10^9$ particles/ml.

‡ ND = < $3 \times 10^8$ particles/ml.

§ The 14-day samples were taken from uninoculated tip leaves.

Table 2. *The percentage of 'long'* virus particles in leaf extracts*

| Days after inoculation | Temperature | | | | |
|---|---|---|---|---|---|
| | 14° | 18° | 22° | 26° | 30° |
| | | | (*a*) Isolate PRN | | |
| 1 | — | 28 | 32 | — | — |
| 2 | 31 | 23 | 26 | 23 | — |
| 3 | 25 | 28 | 22 | 23 | — |
| 6 | 12 | 23 | 21 | 18 | — |
| 14† | — | 22 | — | — | — |
| | | | (*b*) Isolate CAM | | |
| 1 | — | — | 32 | 18 | 19 |
| 2 | — | 41 | 37 | 21 | 17 |
| 3 | 52 | 41 | 40 | 22 | 16 |
| 6 | 35 | 30 | 36 | — | — |
| 14† | 22 | 20 | 20 | 26 | — |

* 'Long' particles of isolate PRN were > 1350 Å and of isolate CAM were > 900 Å in length.

† The 14-day samples were from uninoculated tip leaves.

*vulgaris* leaves Harrison (1956) found that increases in lesion number above 1 per cm.$^2$ did not affect the amount of virus detected in leaf extracts. In our experiments this figure was probably considerably exceeded at the lower temperatures, but at the higher temperatures the fewer infections per leaf may well have limited the amount of virus produced.

Scattered necrotic areas formed at 14° in the uninoculated leaves of plants infected with isolate PRN, but such leaves contained fewer virus particles at any temperature than inoculated leaves. At 18° there were $2 \times 10^{10}$ particles/ml. and at 14°, 22° and 26° only trace amounts. Isolate CAM, however, produced many more particles in the uninoculated tip leaves, 19, 370, 430, 42 and about 1 (all $\times 10^{10}$/ml.) respectively at 14°, 18°, 22°, 26° and 30°.

The concentrations of long particles followed the same general pattern as described for total particle concentrations (Table 1). Indeed, temperature had little effect on the ratio of long to short particles (Table 2). No effect of temperature was detectable with isolate PRN, and with isolate CAM there was only a slight tendency for the proportion of long particles to decrease at the higher temperatures. There was a tendency for the proportion of long particles of each isolate to decrease with increasing time after inoculation, but more remarkable was the constancy of this proportion, despite the range of temperatures and sampling times used. Isolate CAM produced a slightly larger proportion of long particles than isolate PRN.

*Effects on relative infectivity*

One day after inoculation with isolate CAM, relative infectivity increased with temperature up to 30°, but infectivity was not detected at 34° (Table 3). As the time

Table 3. *The relative infectivity of leaf extracts*

| Days after inoculation | Relative infectivity at: 14° | 18° | 22° | 26° | 30° | 34° |
|---|---|---|---|---|---|---|
| | | | (*a*) Isolate PRN | | | |
| 1 | 4·3* | 47 | 91 | 1·5 | 0·02 | ND |
| 2 | 110 | 240 | 91 | 3·8 | T | ND |
| 3 | 210 | 230 | 69 | 7·9 | ND | ND |
| 6 | 250 | 47 | 38 | 0·3 | ND | ND |
| 14† | 5 | 1·4 | 0·35 | 0·03 | ND | ND |
| | | | (*b*) Isolate CAM | | | |
| 1 | 0·02 | 0·09 | 1·3 | 5·3 | 5·9 | ND |
| 2 | 0·8 | 5·7 | 30 | 4·2 | 5·1 | ND |
| 3 | 1·5 | 49 | 36 | 2·8 | 0·12 | ND |
| 6 | 42 | 91 | 70 | 0·90 | 0·01 | ND |
| 14† | 6·5 | 91 | 150 | 35 | 0·42 | ND |

* Relative infectivity = reciprocal of dilution of sample calculated to give the same number of lesions per half-leaf of French bean as the standard diluted 1/125. T (trace) = < 0·01; ND = no infectivity detected.

† The 14-day samples were taken from uninoculated tip leaves.

after inoculation increased, the optimum temperature for accumulation of infective particles decreased to 22° after 2 days and to 18° after 3 to 6 days. The factor by which infectivity increased between 1 and 6 days decreased from 2100 at 14° to about 1000 at 18° and 50 at 22°; during the same interval infectivity *decreased* at 26° and 30° by factors of about 60 and 600 respectively. At 14 to 22°, infectivity was greater after 6 days than earlier, but at 18 to 22° it increased little between 3 and 6 days. At 26 to 30°, infectivities were greatest 1 day after inoculation. The pattern was essentially

similar with isolate PRN, except that the equivalent temperatures again were 4 to 6° below those for isolate CAM. One day after inoculation with isolate PRN infectivity increased with increase of temperature up to 22° but decreased with further increase of temperature up to 30°. Infectivity was never obtained from leaves kept at 34°. The optimum temperature for accumulation of infective virus decreased from 22° at 1 day after inoculation, to 18° at 2 or 3 days and to 14° at 6 days. Between 1 and 6 days after inoculation infectivity increased by a factor of about 60 at 14°; at 18° it increased and then decreased to its initial level, and at 22° and 26° it decreased by 6 days to 0·4 and 0·2 respectively of the 1-day values.

The infectivities of the 14-day samples again show the greater tendency of isolate CAM than of PRN to become systemic. The optimum for isolate PRN was 14° and for CAM was 22°, the same as found with inoculated leaves 6 days after infection.

*Effects on specific infectivity*

Because only the long particles cause lesions in *Phaseolus vulgaris* leaves, specific infectivity was calculated by dividing the relative infectivity of the samples by the

Table 4. *The specific infectivity of 'long' particles**

| Days after inoculation | Specific infectivity at: 14° | 18° | 22° | 26° | 30° |
|---|---|---|---|---|---|
| | | (*a*) Isolate PRN | | | |
| 1 | — | 45 | 51 | — | — |
| 2 | 42 | 61 | 15 | 22 | — |
| 3 | 45 | 56 | 22 | 19 | — |
| 6 | 74 | 16 | 16 | 1·7 | — |
| 14† | — | 30 | — | — | — |
| | | (*b*) Isolate CAM | | | |
| 1 | — | — | 1·9 | 6·8 | 11 |
| 2 | — | 1·6 | 3·9 | 1·4 | 4·7 |
| 3 | 2·9 | 4·9 | 3·6 | 1·6 | 0·02 |
| 6 | 8·1 | 7·0 | 6·5 | — | — |
| 14† | 16 | 12 | 17 | 32 | — |

* Specific infectivity $= \frac{\text{relative infectivity} \times 10^{11}}{\text{no long particles/ml.}}$.

† The 14-day samples were from uninoculated tip leaves.

concentration of long particles they contained (Table 4). Specific infectivity differed considerably between samples, was several-fold greater for isolate PRN than for isolate CAM but followed the same general pattern with both. At 14°, where the amount of virus increased throughout the six days, the specific infectivity increased with time. By contrast, at 26 to 30°, where virus-particle concentration decreased during the latter part at least of the period 1 to 6 days after inoculation, the specific infectivity also decreased greatly. Thus, with isolate CAM, specific infectivity increased with time at 14 to 22° but at 26 to 30° decreased from day 1 onwards. Likewise with isolate PRN, specific infectivity increased up to day 6 at 14°, but at 18° it increased up to day 2 and then decreased, and at 22 to 26° it decreased from day 1 on. Specific infectivity occasionally decreased before particles became fewer. For example, at 22° the concentration of PRN long particles increased between 1 and 2 days after inocula-

tion whereas specific infectivity decreased; also, at 26° and 30° the concentration of CAM long particles increased between 1 and 2 days after inoculation, but specific infectivity declined. When particles became fewer, relative infectivity decreased more than particle number. This is seen at 26° between 3 and 6 days after inoculation with isolate PRN, and at 30° between 2 and 3 days after inoculation with isolate CAM. Isolate CAM had a greater specific infectivity when obtained from systemically infected than from inoculated leaves.

## DISCUSSION

Although the tobacco rattle viruses responded strikingly to temperature changes, the ratio of numbers of long to short particles differed little at different times (see also Semancik & Kajiyama, 1967) or temperatures after inoculation. This suggests that, when infection spreads through the leaf in the conditions of our experiments, the long and short particles interact efficiently at ratios of one long particle to 1 to 5 short ones. Indeed both kinds of particle accumulate faster than particles of many other plant viruses. For example, it is unusual to find that tissue extracts are at their most infective when made only 1 day after inoculation, as happens with isolate PRN at 22°. With a tobacco necrosis virus in *Phaseolus vulgaris* leaves (Harrison, 1956), infective particles at first accumulated far slower than did those of the tobacco rattle viruses in tobacco leaves. However, the tobacco necrosis virus continued to accumulate during the second and third days after inoculation, when the tobacco rattle viruses increased only slowly, or decreased, in amount. Also, whereas 22° was optimal for accumulation of the tobacco necrosis virus at either 1, 2 or 3 days after inoculation, the optimum for the tobacco rattle viruses depended greatly on the time of sampling. These differences suggest that *in vivo* inactivation of virus is more important with the tobacco rattle viruses than with the tobacco necrosis virus.

With several small isometric plant viruses and with the sausage-shaped alfalfa mosaic virus, specific infectivity decreases when infected plants are kept for increasing times after inoculation, in some instances at or above 30° and in others at lower temperatures (Kassanis, 1952; Matthews & Lyttleton, 1959; Kuhn & Bancroft, 1961; Kodama & Bancroft, 1964; Gillaspie & Bancroft, 1965; Kuhn, 1965). Specific infectivity of these viruses was determined by relating relative infectivity to serological or spectrophotometric measurements, and there are few comparisons between infectivity assays and particle counts by electron microscopy. When such comparisons have been made, as with the elongated potato *X* virus (Rochow, Ross & Siegel, 1955), little difference in specific infectivity was found between samples, and until recently there was little evidence that the specific infectivity of rod-shaped or filamentous plant viruses changes in the same way as does that of the small isometric viruses and alfalfa mosaic virus. However, Semancik & Kajiyama (1967) reported changes in specific infectivity (infectivity per standard E260 reading) that differ for different isolates of tobacco rattle virus. They purified two Californian isolates from whole *Nicotiana clevelandii* plants at intervals up to 35 days after inoculation. The plants were kept at 23 to 27°. The specific infectivity of one isolate changed little, whereas that of the other behaved like the specific infectivity of isolates PRN and CAM. It increased slightly when the infectivity of the preparations increased, and later, when infectivity decreased, the specific infectivity decreased to a third or a quarter of the earlier value.

Lebeurier & Hirth (1966) report a somewhat similar phenomenon with tobacco

mosaic virus grown at different temperatures. They found that the specific infectivity of tobacco mosaic virus antigen cultured in leaf discs at 35° was only a third of that of antigen cultured in discs at 24°; they showed that this difference mainly reflected an accumulation at 35° of virus protein that aggregated to form virus-like particles but was free from RNA. Accumulation of such protein seems unlikely to cause the tenfold or larger decreases in specific infectivity we found at the higher temperatures, because tobacco rattle virus protein does not readily aggregate to form virus-like particles that would be counted by electron microscopy (Offord, 1966).

Lebeurier & Hirth (1966) also show that the amount of tobacco mosaic virus antigen in leaf discs kept at 28° halved between 10 and 15 days after inoculation, but it is not clear how much of this decrease can be attributed to breakdown of RNA-free virus protein and how much to loss of virus nucleoprotein. With the tobacco rattle viruses there can be little doubt that virus nucleoprotein is broken down in leaves. At 30° the numbers of particles of isolate CAM found in extracts dropped more than 100-fold between two and six days after inoculation, and infectivity decreased by an even greater factor. The mechanism of *in vivo* inactivation is not known. The curves in Fig. 1 seem explicable either (1) by inactivation of virus at a constant rate dependent on temperature,with the rate of virus synthesis decreasing with increasing time after inoculation, or (2) by the mediation of virus-inactivating systems of the host that are stimulated by virus infection and operate either with or without a slowing of virus synthesis.

The decrease in specific infectivity at high temperatures shows that infectivity is lost before the particles are disrupted. With broad bean mottle virus (Kodama & Bancroft, 1964) decrease in specific infectivity is associated with breakage of the RNA strand within the virus particle. With tobacco rattle virus, the numbers of long particles were used to calculate specific infectivity, so a similar association cannot hold unless breakage of the RNA strand fails to break the particles, or the particle breakage is so close to one end that it is not obvious. Further work is needed to see whether high temperature has subtle effects on particle length.

Several interesting points are brought out by comparing the behaviour of the two tobacco rattle viruses. The lower temperature optima of isolate PRN than of CAM possibly reflect the adaptations of the two viruses to the differing field temperatures in the localities where they were obtained—eastern Scotland and Campinas, Brazil, respectively. Among plant viruses, isolate PRN has unusually low optimum temperatures but many of the other viruses for which there is information have been studied because they occur in tissues in large amounts and, in this at least, they are not representative of most plant viruses. Indeed, we predict that many more of the viruses prevalent in cool temperate regions will be found to accumulate optimally at or below 18°.

The fact that isolate PRN killed many leaf cells, and isolate CAM did not, seems to have had surprisingly little effect on the response of the viruses to temperature. Both accumulated rapidly at first and then more slowly. Also, although fewer particles of isolate PRN were lost at 26° between 3 and 6 days after inoculation than of isolate CAM, this difference is not related to a difference in host reaction, for neither virus caused necrotic lesions at 26°. Thus isolate PRN, although having a lower temperature optimum than isolate CAM, seems the more stable *in vivo*, as it is when stored in *vitro*.

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