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Infectivity Changes in Preparations from Plants Infected with Tobacco Mosaic Virus

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Replication of tobacco mosaic virus or its nucleic acid in cell-free preparations from plants has recently been claimed. Some of the evidence supporting such claims has been based on increases in infectivity measured by inoculating assay plants with these preparations or phenol extracts from them (1–5). During attempts to repeat this work it was observed that such increases in infectivity also occurred in conditions precluding the replication of the virus or its nucleic acid.

Cell-free preparations were made from leaves of *Nicotiana tabacum*, type White Burley, var. Judy's Pride, 5 to 6 days after they were inoculated with tobacco mosaic virus. The leaf tissue was ground with pestle and mortar adding per gram of leaf 1 ml. of buffer containing 0·04 M-sucrose, 0·05 M 2-amino-2-hydroxymethylpropane-1,3-diol (tris) adjusted to pH 7·5 with HCl, and 0·005 M-$MgCl_2$. The mush was strained through two layers of cheesecloth, and the filtrate centrifuged for 1½ hr at 106,000*g*, or for 3½ hr at 42,000*g* (all operations at 0 to 4°). The pellet was resuspended in one-tenth volume of fresh buffer. This suspension was divided into equal parts, to each of which reagents were added bringing the solution to these final concentrations: 0·05 M-tris (pH 7·5); 5 mM-$MgCl_2$; 15 mM-KCl; 3 mM-2-mercaptoethanol; 0·1 g./ml. bentonite (or 0·008 g./ml. spermine); 0·04 M-sucrose; 10 mM phosphoenol pyruvate; 20 μg./ml. pyruvate kinase (specific activity 125 units/mg., C. F. Boehringer and Soehne, Mannheim, Germany); and 100 μg./ml. of each ribonucleoside-5′-triphosphate ATP, GTP, UTP, and CTP. This final solution contained an amount of leaf extract equivalent to 5 g. of the original leaf material per ml.

Immediately after adding the reagents, those samples to be incubated were brought to a temperature of 20 to 22°. The control samples were shaken at 0 to 4° with an equal volume of water-saturated phenol, and the suspension was centrifuged. The clear water layer was drawn off and extracted three times with ether to remove phenol. This procedure was also applied to the incubated samples after 2 to 3 hr at 20 to 22°. The preparations of nucleic acid so obtained were immediately used to inoculate half-leaves of *N. tabacum* var. Xanthi, 6 to 8 weeks old, which had been sprinkled with carborundum powder. Usually each inoculum was applied to 6 to 12 half-leaves; each occurred an equal number of times on each plant, and an equal number of times on each leaf position.

Preparations of nucleic acid instead of the untreated incubation mixture itself were used to inoculate the assay plants because if the virus nucleic acid increased, but not whole virus particles, this increase might be masked in the assay by the much greater infectivity of the whole virus particles already in the extract. The use of nucleic acid preparations as an inoculum insured that any absolute increase in total virus nucleic acid would be observed as a proportional increase in infectivity.

Infectivity increased in incubation mixtures containing a complete complement of phosphoenol pyruvate, pyruvate kinase, and all four ribonucleoside triphosphates

(Table 1), but this increase did not depend on the presence of all four triphosphates, contrary to previous reports (2 to 5). Omission of other components of the system also did not prevent the increase. Increases in infectivity did not occur in all experiments, and in one there was a decrease under the same conditions as those giving increases in other experiments (Table 1). The lack of dependence of the results on the presence of ribonucleoside triphosphates suggested that the increase in infectivity might be caused by some phenomenon other than an increase in virus or virus nucleic acid. One sample was therefore left at 2° during the period that a second was incubating at 22°; a third was frozen and then thawed when the incubation at 2° and 22° ended. Preparations of nucleic acid were immediately made from all three and inoculated to the assay plants. Incubation at 2° increased infectivity as much as incubation at 22°, and freezing and thawing increased infectivity even more (Expt 6, Table 1).

Table 1. *Numbers of lesions produced by extracts from infected plants*

Expt no.	Component(s) omitted	Mean no. of lesions per half-leaf: Controls (no incubation)	Mean no. of lesions per half-leaf: Incubated samples	Conditions of incubation
1	Pyruvate kinase	17·3	41·7	3 hr at 21°
	Pyruvate kinase CTP	30·0	133·0	
2	None	0·5	14·5	3 hr 40 min. at 21°
	CTP	1·7	14·9	
	CTP, ATP, GTP, UTP	2·7	24·0	
3	CTP, ATP, GTP, UTP (spermine substituted for bentonite)	23·3	49·0	2 hr at 20°
	CTP, ATP, GTP, UTP, mercaptoethanol	2·7	40·7	
4	CTP, ATP, GTP, UTP	2·5	2·2	2 hr 40 min. at 20°
	CTP, ATP, GTP, UTP, pyruvate kinase	9·0	1·9	
	CTP, ATP, GTP, UTP and phosphoenol pyruvate	12·5	3·3	
5*	CTP, ATP, GTP, UTP	< 17	> 100	3 hr at 21°
			> 100	
	CTP, ATP, GTP, UTP	< 21	> 100	Freezing and thawing
			> 100	3 hr at 2°
6	CTP, ATP, GTP, UTP	3·5	42·5	3 hr 15 min. at 22°
			123·8	Freezing and thawing
			45·3	3 hr 15 min. at 2°

* In this experiment the leaves were damaged by carborundum; it was not possible to count the lesions accurately, but it was obvious that there had been an increase.

These results do not disprove that tobacco mosaic virus or its nucleic acid can replicate in cell-free extracts; nor do they disprove that replication had occurred in some of my experiments or those of others. However, the fact that infectivity increased when the virus or its nucleic acid had not multiplied (e.g. after freezing and thawing) suggests that such increases are not good evidence for *in vitro* virus replication. Keeping the preparation cold or freezing and thawing it may allow more of the virus nucleic acid already present to be extracted; if this is so, the same explanation may apply to the results of incubation at 22°.

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