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The Behaviour of some Plant Viruses after Exposure to Ultraviolet Radiation

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SUMMARY: Preparations of tobacco mosaic virus (TMV) inactivated by ultraviolet radiation interfered slightly with infection by active tomato bushy stunt (BSV) and Rothamsted tobacco necrosis (RTNV) viruses, and much more so with active TMV. Similarly, inactivated RTNV interfered slightly with infection by TMV and more so with active RTNV. In contrast, inactivated BSV did not affect the numbers of lesions produced by active virus preparations.

The residual infectivity of irradiated preparations of RTNV and BSV was greater when inoculated plants were exposed to light than when they were kept in the dark. This occurs because of some light-sensitive mechanism in the host cells, and exposing the irradiated virus preparations to visible light did not affect their infectivity. Irradiated preparations of TMV had the same residual infectivity whether plants were placed in the light or dark after inoculation.

Although the three viruses have particles of different sizes and shapes, the course of inactivation by ultraviolet with each approximated closely to that of a first-order reaction.

Exposing preparations of some plant viruses to ultraviolet radiation destroys their infectivity without producing any gross changes in their physico-chemical or serological properties (Stanley, 1936; Bawden & Pirie, 1938*a*, *b*). Studies on the course of inactivation have led to the conclusion that it follows the course of a first-order reaction, which has sometimes been taken to indicate that inactivation is caused by a 'single hit'. The conclusion that infectivity decreases exponentially with time of irradiation has been reached whether workers have measured residual infectivity by assuming that numbers of local lesions are proportional to the concentration of infective virus (Price & Gowen, 1936; Lea & Smith, 1940), an assumption known to be invalid since Holmes (1929) first described the use of local lesions for quantitative work, or whether they have used some other method (Oster & McLaren, 1950). Despite the agreed conclusion, therefore, the problem cannot be taken as settled, particularly as deviations from an exponential function have not been tested to see whether they fall within experimental error. Because of this uncertainty, we have re-examined the course of inactivation, although this was not the main purpose of the experiments we describe. These were done primarily to see if phenomena described with irradiated preparations of some bacteriophages are shown by plant viruses. Experiments were made to test for three phenomena, namely: (1) whether inactivated virus interferes with the establishment of active virus, a phenomenon that has been demonstrated with several animal viruses (Henle, 1950) as well as with bacteriophages (Luria & Delbrück, 1942; Kleczkowski & Kleczkowski, 1953); (2) whether irradiated preparations are proportionally more infective when concentrated than when dilute, a

phenomenon described and called 'multiplicity reactivation' by Luria & Dulbecco (1949); (3) whether visible light affects the infectivity of irradiated preparations, a phenomenon described and called 'photoreactivation' by Dulbecco (1950).

MATERIALS AND METHODS

Purified preparations of three viruses were used; those of tobacco mosaic virus (TMV) and tomato bushy stunt virus (BSV) were made by precipitation methods and those of the Rothamsted culture of a tobacco necrosis virus (RTNV) by differential ultracentrifugation.

The test plants were those that react with countable necrotic local lesions, *Nicotiana glutinosa* with TMV and BSV, and French bean (*Phaseolus vulgaris* var. Prince) with RTNV. Inocula to be compared were rubbed as evenly as possible over the upper leaf surfaces with the forefinger. In most tests, eight inocula were compared; these were applied to half-leaves distributed among the test plants so that errors arising from differences in susceptibility between plants, or, with *Nicotiana glutinosa*, between leaves occupying different positions on the stem, could be eliminated (Kleczkowski, 1950). Each inoculum was rubbed over twelve half leaves. Sometimes twelve inocula were compared on *N. glutinosa*; then each was applied to nine half leaves, nine plants being used, each with six leaves. In some experiments to find the effect of exposing inoculated plants to visible light, inoculum of irradiated and control virus could be used at only one concentration. No exact quantitative conclusions were drawn from comparisons of lesion numbers obtained in such experiments, but by suitably adjusting the concentration of the two inocula qualitative effects were clearly obvious. Whenever results were analysed statistically, the numbers of lesions were transformed according to the formula $y = \log_{10}(x+5)$, in which x is the number of local lesions produced per half leaf, and analyses of variances were done on the transformations (Kleczkowski, 1949).

Two sources of ultraviolet radiation were used. One, an 'Osira' 80 W. lamp made by the General Electric Company Ltd., with its glass envelope removed, gives a polychromatic radiation. The other, a low-pressure mercury-discharge lamp made by the Thermal Syndicate Ltd., fitted with a chromium-plated cylindrical reflector, gives a radiation 99 % of which is of wavelength 2536 A. No qualitative differences were noticed between the behaviour of the two lamps in any of our experiments. Virus solutions were irradiated as layers 0·14 cm. deep in Petri dishes, at a distance of 20 cm. from the lamps. In these conditions the intensity of radiation with the low-pressure lamp was 870 μW./sq.cm. The dishes were rocked during the whole exposure to radiation and this is assumed to have ensured that all virus particles had equal opportunities to absorb the same amount of radiation.

EXPERIMENTAL

Interference between active and inactivated virus

The ability of virus inactivated by ultraviolet to interfere with infection by active virus was tested by comparing the relative infectivities of solutions of control virus diluted in water with those similarly diluted in solutions of

inactivated virus at various concentrations. Some typical results are recorded in Table 1, which shows the sums of actual numbers of local lesions produced and the sums of values transformed for statistical analyses. As found with different bacteriophages (Luria & Delbrück, 1942), inactivated preparations of different plant viruses also differ in their behaviour. Inactivated BSV had no effect on the numbers of lesions produced by active preparations of either BSV or TMV, whereas inactivated preparations of TMV and RTNV decreased the numbers produced by all the active viruses with which they were mixed. However, inactivated TMV interfered much more with active TMV, and inactivated RTNV with active RTNV, than either did with other viruses.

Table 1. *Interference of inactive with active viruses*

Exp. no.	Contents of inocula (mg./l.) Untreated virus	Inactivated virus*	Total numbers of lesions on twelve half-leaves	The sums of twelve transformed numbers of lesions†	Host plants
1	2 TMV	5000 TMV	270	17·11	*Nicotiana glutinosa*
	2 TMV	5000 BSV	630	20·34	
	2 TMV	—	632	20·52	
2	0·2 TMV	5000 TMV	30	—	
	0·2 TMV	5000 BSV	104	—	
	0·2 TMV	—	119	—	
3	5 BSV	5000 TMV	195	15·30	
	5 BSV	5000 BSV	359	17·17	
	5 BSV	—	371	17·51	
4	0·5 BSV	5000 TMV	38	—	
	0·5 BSV	5000 BSV	48	—	
	0·5 BSV	—	48	—	
5	1 TMV	4000 TMV	207	15·58	
	1 TMV	4000 RTNV	401	18·21	
	1 TMV	—	468	19·05	
6	0·2 TMV	4000 TMV	52	11·19	
	0·2 TMV	4000 RTNV	139	13·94	
	0·2 TMV	—	196	15·06	
7	1 RTNV	4000 TMV	440	19·12	French bean
	1 RTNV	4000 RTNV	272	17·19	
	1 RTNV	—	580	20·47	
8	0·2 RTNV	4000 TMV	107	—	
	0·2 RTNV	4000 RTNV	44	—	
	0·2 RTNV	—	193	—	

* The viruses were irradiated at 1 % solutions. The times of irradiation were: TMV, 3 hr., BSV, ½ hr., and RTNV, 1 hr.

† The transformation was $y = \log_{10}(x+5)$, where x's are the numbers of lesions per half-leaf.

Statistically significant differences between the sums of transformed numbers for different levels of probability (P) obtained from a table of 'Student's' distribution of t.

Exp. no.	P 0·05	0·02	0·01	0·001
1	1·76	2·12	2·38	3·16
3	1·88	2·27	2·55	3·43
5	2·25	2·70	3·03	4·01
6	1·77	2·13	2·38	3·16
7	1·29	1·56	1·74	2·31

The interpretation of these results is uncertain. Many substances interfere with infection by plant viruses, and several are known that are powerful inhibitors of TMV when used at much smaller concentrations than 0·5 mg./ml., the smallest at which inactivated TMV had any demonstrable effect. These substances do not act specifically against individual viruses, but the extent of their inhibitory power does vary with the identity of the host plant. The mechanism of inhibition is undetermined, but there is growing evidence that the inhibitors act by altering the host-cell metabolism rather than by directly affecting the virus particles (Gupta & Price 1950, 1952; Bawden & Freeman 1952). It is reasonable to assume that the inhibiting effect of inactivated TMV on BSV and RTNV is analogous to that of these other substances. There is, however, evidence of an additional and larger effect operating specifically between inactivated and active TMV and between inactivated and active RTNV. At first sight this suggests that two different mechanisms may be involved, but this is not necessarily so. Both the specific and unspecific interference could result from the same cause, and our results are most simply explained by postulating that inactivated TMV and RTNV, but not inactivated BSV, affected the metabolism of leaf cells into which they were introduced, so that the cells became less favourable for any virus to become established but particularly so for one resembling the initial stimulus. We have no evidence that the inactivated plant viruses do affect cellular metabolism, but, by analogy with results from tests with irradiated bacteriophages, it is reasonable to assume they do. That bacteria can be changed by absorbing inactivated particles of some bacteriophages is shown by their subsequent inability to divide (Luria & Delbrück, 1942; Kleczkowski & Kleczkowski, 1953).

Interpreting similarities and differences between results with bacteriophages and with plant viruses is complicated because the initiation of infection with the two calls for such different conditions. To gain infection with plant viruses, host cells need to be wounded and other materials than virus particles can presumably also enter through the wounds. In contrast, bacteriophages infect uninjured bacteria and only bacteriophages and other materials that are specifically absorbed by bacteria can enter. This difference may explain the fact that a small non-specific inhibition is produced by irradiated TMV and RTNV, whereas no such effect has been observed with bacteriophages, with which interference by inactivated particles depends on the susceptibility of the bacteria to the particular phage (Kleczkowski & Kleczkowski, 1953). Another difference between the two is that, if inactivated particles interfere with infection by active particles of the same phage, they also seem to interfere equally with infection by other phages to which the bacterium is susceptible (Luria & Delbrück, 1942), whereas in *N. glutinosa*, which is a host plant for both TMV and RTNV, inactivated RTNV has much less effect on TMV than inactivated TMV. This difference is possibly explained by the fact that susceptible bacteria are more affected by the entry of inactivated virus particles, as is suggested by the inability of the cells to multiply, than are leaf cells of higher plants.

Inactivated particles of coli phages lose their ability to interfere with infec-

tion by active particles if they are exposed to too much ultraviolet (Luria & Delbrück, 1942). We have made no special tests for this phenomenon with plant viruses, but within the range of minutes to hours that we have irradiated preparations of TMV and RTNV inactivated particles seem equally effective. Interference occurs with preparations irradiated well beyond the point at which any residual infectivity is demonstrable and in those irradiated so that they retain about 1 % of the original infectivity. It is the only phenomenon suggesting any interaction between individual virus particles that we have noted, and we found nothing to indicate that particles inactive singly could together become active. That such a thing might happen was suggested by Luria & Dulbecco (1949) to explain their results when irradiated preparations of some coli phages were mixed in various proportions with bacteria before being diluted and plated. They found that the numbers of plaques produced by irradiated preparations increased with increase in the ratio of the preparation to bacterial cells in the mixture before dilution.

The procedure used with bacteriophage is obviously inapplicable to plant viruses, but if multiple infections with particles inactive singly lead to virus multiplication, this should be detectable by comparing the dilution curves given by partially inactivated preparations with those given by unirradiated preparations. As the concentration of virus in the inoculum increases, the mean number of particles entering an infection site will be expected to increase, an expectation that is supported by the ability of concentrated preparations of inactivated TMV to interfere with the multiplication of active particles. By plotting the numbers of local lesions against logarithms of virus concentration, therefore, the phenomenon of multiplicity reactivation should show by the irradiated preparations giving a steeper curve than control preparations.

Tests with TMV, BSV and RTNV, irradiated for various times to give residual activities between 0·1 and 10 % of the original preparations, gave no evidence that particles inactive singly could cause infection when acting jointly. All the irradiated preparations gave dilution curves of the same form as those given by control virus preparations, until the concentration of virus in the irradiated preparations reached about 0·1 %. Then, instead of getting the steeper curve to be expected with multiplicity reactivation, the irradiated preparations gave flatter curves than the control preparations, because at this level the inactivated particles began to prevent infection with the active ones.

To account for the proportionally greater infectivity of concentrated preparations of their irradiated bacteriophages, Luria & Dulbecco (1949) suggested that particles contain several activities or 'genes' each of which can be destroyed singly, and that activities in different particles can replace one another, so that two particles in which different activities are destroyed still supply the full complement needed in a bacterium for phage production. Were this interpretation correct, it seems likely that structures of this type would be typical rather than restricted to a few viruses. The phenomenon of proportionally greater infectivity at high concentrations, however, seems to occur rarely, for it was not found with all coli phages (Luria & Dulbecco, 1949), with a staphylococcal phage (Price, 1950), with two phages of *Rhizobium* sp.

(Kleczkowski & Kleczkowski, 1953), or with the plant viruses we have studied. When irradiated preparations of bacteriophages at different dilutions are mixed with bacteria, it is not only the ratio of phage particles to bacterial cells that alters, but also the ratio of everything else in the preparation. Ultraviolet may change other components than virus particles, and infection with the coli phages studied by Luria & Dulbecco may be facilitated when these changed components exceed a critical concentration.

Effects of visible light on infectivity

Before making detailed measurements on rates of inactivation, tests were done to see whether such measurements would be affected by the treatments to which the irradiated virus preparations and inoculated plants were subjected. Kelner (1949, 1951) has shown that the proportion of irradiated fungal spores and bacteria that multiply is greater if cultures are exposed to visible light than if they are kept in darkness, and Dulbecco (1950) that the residual activity of irradiated bacteriophage is greater if the inoculated bacteria are illuminated than if kept dark. With two of the plant viruses, RTNV and BSV, we obtained results similar to those obtained with the bacteriophages. When irradiated preparations of these two viruses were exposed to visible light *in vitro*, their activity was unaffected, but when plants were exposed to light after inoculation the residual infectivity was increased. TMV behaved differently, and exposing either the irradiated virus preparations themselves or the inoculated plants to visible light did not increase the residual infectivity.

Various factors complicate experiments to test the effect of exposing inoculated plants to light on the infectivity of irradiated virus preparations. There is no direct proportionality between numbers of local lesions and concentration of infective virus, individual plants may produce widely different numbers of lesions when rubbed with the same inoculum, and the numbers produced by unirradiated inocula depend on the treatment of the plants, including their illumination. Conclusions can be drawn only from comparisons between irradiated and unirradiated inocula applied to similarly treated plants in experiments statistically designed.

Table 2 shows the results of one experiment with each of the three viruses, which were irradiated so that their residual infectivity was about 0·5 % of the original when the host plants were kept in darkness after inoculation. Control virus preparations were used at a hundred times the dilution of the irradiated preparations, so that both produced numbers of lesions that did not differ greatly. Tests were made with plants, some of which had been in darkness for a day before they were inoculated and some of which had been maintained under normal glasshouse conditions. After inoculation each batch of plants was divided into two, and one lot was kept in darkness for a day whereas the other was kept under ordinary glasshouse conditions. The experiments with all three viruses confirm previous results (Samuel & Bald, 1933; Bawden & Roberts, 1948) that keeping plants in darkness before inoculation increases their susceptibility, and they show that the numbers of lesions produced by irradiated and unirradiated inocula are increased equally by this treatment.

A period in the dark after inoculation with unirradiated inocula had less effect, but, as also found previously (Bawden & Roberts, 1948), usually decreased the numbers of lesions. With TMV the decrease was the same with both irradiated and unirradiated inocula, so that the ratio between the numbers of lesions produced by the two inocula was similar whether the plants were illuminated or not. By contrast, the irradiated inocula of BSV and RTNV produced fewer lesions than unirradiated inocula when the inoculated plants were put in the dark, but more when the plants were put in the light.

Table 2. *Effects of exposing host plants to daylight before and/or after inoculation with untreated and with ultraviolet irradiated virus preparations*

		RTNV on French bean		BSV on *N. glutinosa*		TMV on *N. glutinosa*	
		Before inoculation		Before inoculation		Before inoculation	
		Dark	Light	Dark	Light	Dark	Light
Dark after	A	24	6·5	19·5	3	23	13
inoculation	B	14·5	2·5	8	0·7	10	8
Light after	A	24	11·5	84	7·5	50	12·5
inoculation	B	41	26	85	9	34	7

The numbers are mean numbers of lesions per leaf obtained on twelve to fourteen half-leaves.

A, untreated virus at 1 mg./l. RTNV, 5 mg./l. BSV, 1 mg./l. TMV.

B, irradiated virus at 100 mg./l. RTNV, 500 mg./l. BSV, 100 mg./l. TMV.

Irradiation: RTNV 0·01 % 1 min. 20 sec.
BSV 0·1 % 1 min. 30 sec.
TMV 0·1 % 7 min.

Light: the plants were exposed to uncontrolled daylight.
Dark: the plants were kept in darkness for 24 hr.

The numbers of lesions produced by irradiated preparations of BSV and RTNV were increased only when plants were exposed to light soon after they were inoculated. The fate of a virus particle, that is, whether it will become established and multiply, is determined within a few hours; particles seem unable to remain dormant in cells if conditions are temporarily unfavourable and then become established 24 hr. later when conditions are made more favourable by exposing the leaves to light. We have made no detailed experiments on the amount of light needed to give the maximum infectivity with irradiated preparations, but most of the increase occurs when plants are exposed for 3 hr. to daylight under glass, when the intensity at leaf level is around 800 f.c. Although to get a response inoculated plants must be exposed to light without delay, the irradiated inocula need not be used immediately. Tests with BSV and RTNV 14 days after irradiation gave the same relative differences between plants kept in the light and dark after inoculation as with tests made within an hour of irradiation.

The conditions in which infection with irradiated BSV and RTNV is favoured are those in which irradiated leaves can also counteract damage to themselves. Bean leaves exposed to ultraviolet remain apparently unharmed if later kept in the light, whereas their epidermal cells die if they are kept in the dark

(Bawden & Kleczkowski, 1952). The mechanism responsible for increasing the infectivity of the irradiated virus preparations is almost certainly the same as that which counteracts the lethal effects of radiation on the cells. Whether it operates by reversing changes in the virus particles caused by ultraviolet, or by making conditions such that damaged virus particles can still function, is unknown. It seems that the host-cell system responsible is one that operates only in light and does not lead to any accumulation of products that themselves counteract the damaging effects of ultraviolet. This is suggested by the fact that plants kept in the light before inoculation produced proportionally as many more lesions when exposed to visible light after inoculation with irradiated viruses as did plants kept in the dark until they were inoculated. The effect of excluding light before inoculation in increasing susceptibility seems a different phenomenon and may occur simply because the removal of photosynthetic products facilitates the entry of particles when leaves are rubbed.

Rates of inactivation

Experiments in which plants inoculated with virus preparations irradiated for various times were separated into two lots, one of which was kept in the light and the other in the dark, showed that the post-inoculation treatment, provided it was kept constant, was unimportant in studying the course of inactivation. With TMV the rate of inactivation was the same whether plants were kept in the light or dark, whereas with RTNV and BSV it was greater by about 20 % when plants were in the dark than when in the light. The effect was constant throughout the course of irradiation and, with these two viruses, exposing the inoculated plants to visible light was quantitatively equivalent to decreasing the dose of ultraviolet by a constant factor. Kelner (1949) described this phenomenon with irradiated bacteria as the 'dose reduction principle', and it also applies to bacteriophages (Dulbecco, 1950; Kleczkowski & Kleczkowski, 1953).

Table 3 shows the results of experiments in which the residual activity of preparations was measured after various times of irradiation and inoculated plants were kept under normal glasshouse conditions. The course of inactivation closely follows that of a first-order reaction, for variations in the values of k, obtained from the equation $\log_{10} r = -0{\cdot}4343kt$, in which r is the residual activity as a proportion of the original and t is the time of irradiation in minutes, in any experiment are small and could fall within chance variations from a constant value. The values of $\log_{10} r$ given in Table 3 were obtained by graphic interpolation from dilution curves and this method does not allow definite conclusions as to whether deviations fall within experimental error. A statistical method will be described elsewhere (Kleczkowski, 1953) for testing the compatibility of lesion counts with different hypotheses, and this has been applied to Exps. 1, 2 and 4 from Table 3. The test showed that the variations in k in Exps. 1 and 4 do not depart significantly from a constant value; they do in Exp. 2, but the deviation is only slight. Thus it can be assumed that the course of inactivation does approximate closely to that of a first-order reaction, which has also been demonstrated with bacteriophages

Table 3. *The course of inactivation of the viruses by ultraviolet radiation*

Ultraviolet irradiation of virus preparations: Concentration of the preparation (%)	Ultraviolet irradiation of virus preparations: Time of irradiation (min.)	Virus content of inoculum (Log_{10} of percentage of concentration)	Nos. of lesions per half-leaf	$\log_{10} r$*	k†
		Exp. 1. RTNV on French bean			
1·0	2·48	−3·0	134	−0·54	0·50
	4·24		100	−0·85	0·46
	6·00		65	−1·12	0·43
	7·77		29	−1·65	0·49
	Unirradiated control	−3·7	114		
		−4·2	59		
		−4·7	27		
		−5·2	8		
		Exp. 2. RTNV on French bean			
0·1	0·58	−3·0	95	−0·89	3·5
	1·00		39·5	−1·41	3·3
	1·42		17·5	−1·75	2·8
	1·83		4·5	−2·26	2·8
	Unirradiated control	−3·7	125		
		−4·2	56·5		
		−4·7	19·5		
		−5·2	5		
		Exp. 3. RTNV on French bean			
0·01	0·50	−3·0	70	−1·00	4·6
	0·87		19	−1·83	4·8
	1·22		3	−2·60	4·9
	1·58		1·7	−3·20	4·7
	Unirradiated control	−3·7	104		
		−4·2	47		
		−4·7	24		
		−5·2	7		
		Exp. 4. TMV on *N. glutinosa*			
0·5	3·85	−2·5	122	−0·62	0·37
	7·70		100	−0·78	0·23
	11·55		53	−1·27	0·25
	15·40		26	−1·80	0·27
0·01	0·575	−2·5	123	−0·62	2·5
	1·150		64	−1·03	2·1
	1·725		12	−2·25	3·0
	2·300		5·5	−2·70	2·7
	Unirradiated control	−3·0	144		
		−3·5	70		
		−4·0	42		
		−4·5	19		
		Exp. 5. BSV on *N. glutinosa*			
0·1	0·45	−2·3	92	−0·73	3·7
	0·77		68	−0·95	2·8
	1·08		31	−1·53	3·3
	1·40		25	−1·73	2·8
	Unirradiated control	−3·0	95		
		−3·5	47·5		
		−4·0	26·5		
		−4·5	16		

* r is the proportion of residual activity of irradiated virus preparations. The values of $\log_{10} r$ were obtained by graphic interpolation from the dilution curve given by unirradiated virus.

† k is obtained from the equation $\log_{10} r = -0{\cdot}4343kt$, where t is time of irradiation in minutes.

(Latarjet & Wahl, 1945, Latarjet & Morenne, 1951; Kleczkowski & Kleczkowski, 1953). This is insufficient evidence to conclude that single quanta of ultraviolet inactivate, and the minute quantum yield, of the order of 4×10^{-5} (Oster & McLaren, 1950), tells against such a conclusion. Inactivation of many biologically active particles by heat also approximates to a first-order reaction, but no one suggests that heat inactivation occurs because of a 'single hit'. Approximation to a first-order reaction means simply that, in constant conditions, there is a constant probability, $p = 1 - e^{-k}$, that a given particle which is still active will become inactive during the next minute.

There is evidence, too, that absorption of ultraviolet can lead to different kinds of changes. With the plant viruses we have noticed only two, loss of infectivity and the production of particles that can infect plants exposed to visible light but not those kept in the dark. With some *Rhizobium* phages (Kleczkowski & Kleczkowski, 1953) and trypsin (to be published), there is evidence of a third change, for the particles remaining active after irradiation are less stable than particles that have not been exposed to ultraviolet radiation. We have failed to detect such an effect during comparable tests with the three plant viruses, but their greater intrinsic stability than either of the bacteriophages or of trypsin may explain the failure. Preparations of the three viruses, irradiated so that their residual infectivity was about 0·5 % of control preparations when tested immediately after irradiation, had the same relative infectivity when again compared with the controls after a week at 18°. This treatment had no obvious effect on the activity of control preparations and any increase in the rate of spontaneous inactivation produced by irradiation would of necessity have had to be considerable to be detected. That irradiation may affect stability is suggested by the increased sensitivity of irradiated preparations of TMV to denaturation by heat (Oster & McLaren, 1950).

Preparations of BSV consist of spherical particles of a uniform size, and those of RTNV of spherical particles of two different sizes, whereas preparations of TMV consist of rod-shaped particles of greatly differing lengths. It is of some interest, therefore, that inactivation of all three viruses should follow the same course. Despite the differences in their tendencies to aggregate, it seems that particles of all three viruses behave as single infective units and not as aggregates of units all of which need to be inactivated individually. If they consisted of such aggregates, the constant probability, p, of inactivation, would be that of single units and not of aggregates; the probability of inactivation during the next minute of an aggregate containing n active units would be p^n, and this would increase as n decreases during the course of irradiation. The infective units in preparations of TMV, then, are not predominantly aggregates containing more than one infective unit. The small particles have been shown to be poorly infective and not to gain infectivity when aggregated linearly (Bawden & Pirie 1945), and it seems probable that few of the large aggregates contain more than one infective unit.

The experiments recorded in Table 3 were made at different times and with plants kept under different light intensities after inoculation, so that no exact

conclusions should be drawn from quantitative differences between values recorded for k. However, the large effect of varying virus concentration is obvious. The higher the concentration, the lower is the relative rate of inactivation, showing that increasing virus concentration produces a proportionately smaller increase in the amount of ultraviolet absorbed, so that less radiation is absorbed per virus particle in a unit of time.

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