

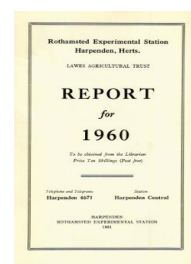
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EFFECTS OF ULTRAVIOLET RADIATION ON PLANT VIRUSES AND ON THE CAPACITY OF HOST PLANTS TO SUPPORT THEIR MULTIPLICATION

BY

A. KLECZKOWSKI

That ultraviolet radiation (UV) can kill plant cells was first recognised by Maquenne & Demoussy in 1909, and that it inactivates a plant virus, namely tobacco mosaic virus, was first demonstrated independently by Mulvania and by Smith in 1926. The emphasis in early research was in comparing the rates of virus inactivation with the rates of killing bacteria, which it was hoped would shed light on the nature of viruses. Mulvania (1926) and Smith (1926) concluded that, as tobacco mosaic virus is much more resistant to UV than are bacteria, it is more comparable to an enzyme than to a bacterium. They compared their results with the virus directly with those obtained by other workers with bacteria, regardless of possible differences in intensities of irradiation or of the fact that the results were obtained in environments that differed widely in their capacities to absorb UV. No significance can, therefore, be attached to the comparisons. This was recognised by Duggar & Hollaender (1934a, b), who made comparisons by irradiating tobacco mosaic virus and different bacteria simultaneously in the same medium. They also found that the virus is much more resistant to UV than all the bacterial species that they tested. However, the considerable variation in susceptibility to UV between bacterial species and even between different stages of growth within one species (Zelle, 1955), and also between different plant viruses (Bawden & Kleczkowski, 1955), make a complete generalisation impossible.

The early trends in research on inactivation of plant viruses by UV very soon assumed the modern character when such problems as action spectra, kinetics, quantum yields, etc., were investigated. By contrast, research on the lethal effect of UV on plant cells progressed little and has not reached a quantitative stage. A new aspect of the subject was opened with the discovery of the phenomenon of photoreactivation, that is that some of the effects of UV on both plants and viruses can be reversed by visible light.

Action spectra

Tobacco mosaic virus is the only plant virus whose action spectrum for inactivation by UV has been determined, first by Duggar & Hollaender (1934a, b) and then slightly corrected (Hollaender & Duggar, 1936). The spectrum of the type strain of the virus is

rather unusual. Plotting relative efficiency of radiation against wavelength gave no peaks. The line rose steadily as the wavelength decreased from 290 m μ , slowly at first and then rapidly below 250 m μ . This has been confirmed recently by Siegel & Norman (1958) and by Rushizky, Knight & McLaren (1960). The action spectrum does not resemble the absorption spectrum of nucleic acid, of virus protein or of the whole virus. The action spectrum of the strain U2 differs from that of the type strain in that, instead of the rise below 250 m μ , it shows a slight drop; it thus has a peak in the vicinity of 260 m μ , and so slightly resembles the absorption spectrum of nucleic acid.

Preparations of the infective nucleic acid from tobacco mosaic virus behave very differently, for Rushizky *et al.* (1960) found that the quantum yields for the inactivation were independent of wavelength, both with and without photoreactivation, so that the action spectrum closely resembles the absorption spectrum of nucleic acid. They also found that reconstituted virus, i.e., the product of recombining separated nucleic acid and protein, behaves like the original virus.

Assuming that infectivity of the virus is a function of its nucleic acid component, the action spectrum of the nucleic acid is therefore drastically modified by the protein component either in the original or in reconstituted virus. This may be because the bonding between protein and nucleic acid protects the nucleic acid from damage by UV (Siegel, Wildman & Ginosa, 1956; McLaren & Takahashi, 1957; Bawden & Kleczkowski, 1959), and the degree of protection may depend on the wavelength. Some kinds of damage by UV in the protein may also interfere with initiation of infection, and such damage may occur predominantly within a particular range of wavelengths. The fact that the action spectrum of the U2 strain of tobacco mosaic virus deviates from that of the isolated nucleic acid less than does that of the type strain, and the fact that nucleic acid in strain U2 is much less protected from damage by UV than is nucleic acid in the "type" strain, suggests that the protection may contribute to the deviation of the action spectrum of the whole virus from that of the isolated nucleic acid.

Kinetics of inactivation

Gowen & Price (1937) and Lea & Smith (1940) concluded that inactivation by UV of tobacco mosaic virus, tomato bushy stunt virus and a tobacco necrosis virus proceeds according to the first-order kinetics, i.e., plotting logarithms of proportions of residual infectivity against doses of irradiation gives straight lines. These authors assumed that residual infectivity over wide ranges is exactly proportional to the numbers of lesions produced on leaves inoculated with irradiated preparations, which is not true in general. Nevertheless, Oster & McLaren (1950), who computed the extent of inactivation by finding dilutions at which irradiated and control solutions of tobacco mosaic virus gave equal numbers of lesions, and Bawden & Kleczkowski (1953), who obtained residual infectivities of irradiated preparations of tobacco mosaic virus, tomato bushy stunt virus and a tobacco necrosis virus, by interpolation from dilution curves obtained by inoculating control virus preparations over a

range of dilutions, also found that inactivation proceeds, approximately at least, according to the first-order kinetics.

Because of the first-order kinetics, most workers concluded that virus particles are inactivated by "single hits", i.e., by single quanta of radiation energy that happen to "hit" regions essential for infectivity. That this may, but need not, be so was pointed out and discussed by Kleczkowski (1960). One fact that throws doubt on the "single-hit" hypothesis, without, however, disproving it, is the extreme smallness of quantum yields. For example, a particle of tobacco mosaic virus absorbs on the average about 25,000 quanta of the radiation of 254 m μ before it is inactivated (Oster & McLaren, 1950; Kleczkowski, 1954).

Photoreactivation

The phenomenon called photoreactivation was discovered by Kelner in 1949, when he found that the proportion of *Streptomyces griseus* conidia that survived UV irradiation was greater when they were subsequently exposed to visible light than when kept in darkness. That the phenomenon extends to some plant viruses, and to leaf cells of such plants as French bean, was found by Bawden & Kleczkowski (1952, 1953). Irradiated viruses do not have their infectivity increased by exposure to visible light *in vitro*. The phenomenon operates through some light-sensitive mechanism in the host cell and shows by the proportion of surviving infective virus being greater when plants are exposed to daylight after inoculation than when they are kept in darkness. Keeping the plants in light or darkness for a period of time before inoculating them with UV-irradiated virus does not affect the apparent proportions of surviving infectivity of the virus.

Whether exposure to visible light reverses the damage caused by UV to plant viruses and to leaf cells, or counteracts the damage in some other way, has yet to be established. However, Lennox, Luria & Benzer (1954), by studying the rates of repeated inactivation and photoreactivation of a bacteriophage inside its host cell, showed that the change caused by UV in the bacteriophages is probably reversed by photoreactivation, and this conclusion probably applies generally.

Photoreactivation increases the residual infectivity of UV-irradiated virus preparations, but does not restore it to its original level. Hence the radiation causes two kinds of damage, only one of which is photoreversible. Kleczkowski (1960) gave evidence that the reversible and irreversible damage occur independently and that the irreversible damage is not a further change in particles already changed reversibly.

The inactivation of plant viruses by UV apparently proceeds according to the first-order kinetics, whether or not photoreactivation operates. Thus, if v is the dose of irradiation, the proportion of residual infectivity with photoreactivation is $p_{\text{light}} = \exp(-k_{\text{light}}v)$ and without photoreactivation $p_{\text{dark}} = \exp(-k_{\text{dark}}v)$, where k_{light} and k_{dark} are constants characteristic for a given virus in a given set of conditions. A ratio $k_{\text{light}}/k_{\text{dark}}$ equal to one means there is no photoreactivation, and any excess of the ratio over one shows the extent of photoreactivation. The ratio differs considerably with

different viruses (Bawden & Kleczkowski, 1955). It may possibly differ even with one virus, depending perhaps on the species of host plant in which photoreactivation is obtained, or on the condition of the host plant, but this has yet to be investigated.

Of several plant viruses now tested, all have shown the phenomenon of photoreactivation except tobacco mosaic and tobacco rattle viruses. Although these two are not photoreactivated when irradiated intact, photoreactivation is shown when their freed nucleic acids are irradiated. (Bawden & Kleczkowski, 1959; Harrison & Nixon, 1959.)

A fact immediately obvious from comparing the rates at which intact tobacco mosaic virus and its free nucleic acid are inactivated by UV is that the nucleic acid is much more resistant to inactivation when it is a part of intact virus than when free (Siegel, Wildman & Ginosi, 1956; McLaren & Takahashi, 1957; Bawden & Kleczkowski, 1959). When the isolated nucleic acid is irradiated, about half of the absorbed radiation energy seems to be concerned with the kind of damage that is reversible by photoreactivation and the other half with the irreversible damage. When intact virus is irradiated, inactivation by radiation energy absorbed per unit of nucleic acid progresses at a rate that is roughly only about one-tenth of that of free nucleic acid, and no photoreactivable damage occurs (Bawden & Kleczkowski, 1959).

The probable reason for the nucleic acid being more resistant to UV when inside the virus than when free is that the type of bonding between the nucleic acid and the protein reinforces the structure of components of nucleic acid. The degree to which nucleic acid is protected by the protein differs with different viruses, and even with different strains of the same virus, as for example with strains U1 and U2 of tobacco mosaic virus (Siegel, Wildman & Ginoza, 1956). Results obtained by Kassanis (1960) suggest that the nucleic acid of a tobacco necrosis virus may be protected only very little or not at all by the protein component. These differences can be explained by assuming differences in the nature of bonding between protein and nucleic acid.

The lack of photoreactivation with tobacco mosaic virus when irradiated intact could have two explanations. The bonding with protein may protect the nucleic acid from the photoreversible kind of damage, while allowing the irreversible kind, or it may prevent visible light from reversing changes caused by UV radiation. That the first is the correct explanation was shown by the failure to obtain photoreactivation when plants were inoculated with the nucleic acid isolated from virus irradiated while intact (Bawden & Kleczkowski, 1959).

Of the plant viruses yet tested, potato virus X showed the phenomenon of photoreactivation most strongly, and using this virus, photoreactivation could be roughly timed. With tobacco plants inoculated with UV-irradiated potato virus X and kept at about 20°, it mattered little whether they were in light or in darkness during the first 30 minutes. After that period had passed, most photoreactivable virus was photoreactivated during about 15 minutes in ordinary daylight, but only when the plants were exposed to light during the next hour. Thus the condition of the virus particles

that are reversibly inactivated by UV changes twice in inoculated leaves. The first change makes them ready for photoreactivation, and, if photoreactivation does not then occur soon, the second change makes them inactive irreversibly (Bawden & Kleczkowski, 1955).

The irradiated free nucleic acid from tobacco mosaic virus behaves differently. Most reversibly inactivated nucleic acid seems to become photoreactivable either immediately or within a few minutes after inoculation to the host plant (*Nicotiana glutinosa*); if it is not photoreactivated within an hour or so, most of it becomes irreversibly inactive (Bawden & Kleczkowski, 1960).

The lethal effect of UV on cells of higher plants can also be reversed by photoreactivation (Bawden & Kleczkowski, 1952; Tanada & Hendricks, 1953; Benda, 1955; Chessin, 1958), but there is no information about the rate at which cells are killed by UV radiation, the extent to which this can be reversed by photoreactivation, the rate of photoreactivation and the effect of time-interval between exposure to UV and to visible light.

Action spectra for photoreactivation of UV-effects on infectivity of plant viruses or on viability of plant cells have not yet been obtained. However, by the use of selective light filters Tanada & Hendricks (1953) found that the lethal effect of UV on cells of leaves of soybean was prevented by light of wavelengths shorter than 450 m μ , and Chessin (1958) found the same with French bean leaves and also with potato virus X. These results fit with those previously obtained with other materials, such as a bacteriophage (Dulbecco, 1950), *Escherichia coli* and *Streptomyces griseus* (Kelner, 1951). The action spectra for photoreactivation of these materials have peaks near 350 or 450 m μ and fall to zero below 300 and above 500 m μ .

Loss of infectivity and structural alteration in virus

The photochemistry of inactivation of plant viruses by UV is still unexplored. All the information shows that irradiation destroys infectivity without causing any gross changes in the structure of the particle. That virus preparations could be inactivated but still retain their ability to crystallise and to react with specific antisera has long been known (Stanley, 1936; Bawden & Pirie, 1938a, b). Oster & McLaren (1950) found that tobacco mosaic virus preparations that had lost more than 98% of their infectivity showed no change in viscosity, sedimentation constant, optical turbidity, isoelectric point, appearance in the electron microscope or UV absorption spectrum. McLaren & Takahashi (1957) also found that infective nucleic acid isolated from tobacco mosaic virus did not alter appreciably either in viscosity or in UV absorption spectrum after it had lost 99.99% of infectivity. All this suggests that neither the protein nor the nucleic acid components are much altered when infectivity is lost.

The structural changes responsible for loss of infectivity are unknown, but as pyrimidines are very much more susceptible than purines to changes by UV, it is reasonable to suspect that loss of infectivity results from damage in pyrimidine residues of the virus nucleic acid. Whether the photoreversible change caused by UV in virus nucleic acid is the reversible hydrolysis in the double bond between 5 and 6 positions in cytosine and uracil, as suggested by

Shugar & Wierzchowski (1958), is still questionable, for it has so far been reversed only by acid, alkali or heat, and whether in suitable conditions it can also be reversed by exposure to visible light still remains to be tested.

Inactivation of the capacity of plants to support virus multiplication

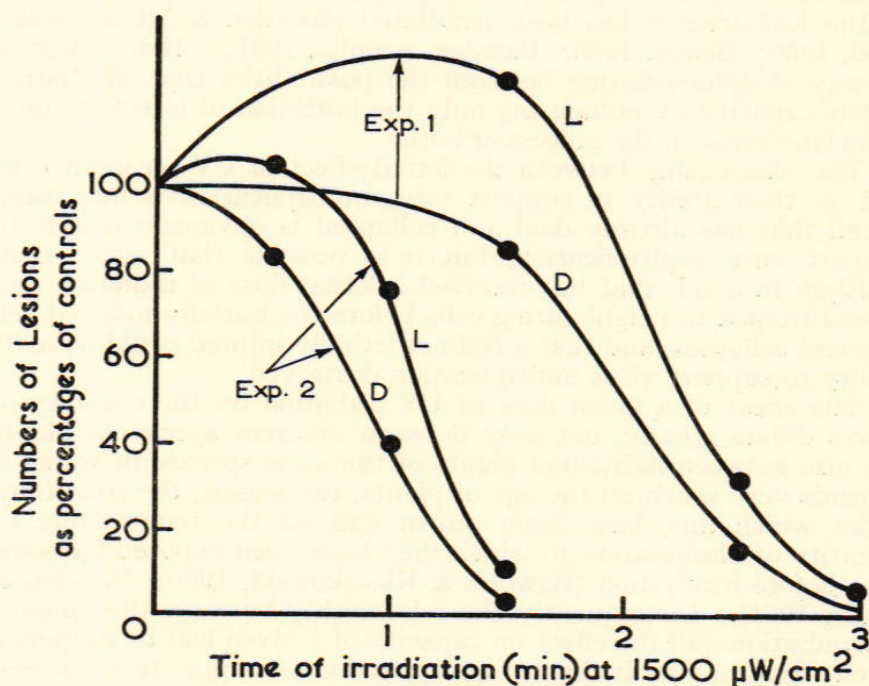
The word "capacity" is used here to mean the ability of a leaf to support multiplication of a virus to the extent of forming local lesions. The only current method of measuring the effect on capacity of exposing a leaf to UV radiation is to observe the effect on numbers of lesions formed by virus preparations that are inoculated to the leaf after it has been irradiated (Bawden & Kleczkowski, 1952, 1960; Benda, 1955; Bawden & Sinha, 1961). Hence there is no way of differentiating between the possibilities that irradiation affects capacity by influencing only the initiation of infection, only some later stage in the process or both.

The relationship between the lethal effect of UV on plant cells and on their ability to support virus multiplication is uncertain. A cell that has already died and collapsed is obviously unable to support virus multiplication, but it is possible that virus could multiply in a cell that has received a lethal dose of radiation and spread from it to neighbouring cells before the initially infected cell dies and collapses, and that a cell not lethally injured could have its ability to support virus multiplication destroyed.

The effect of a given dose of UV radiation on the capacity of leaves differs greatly, not only between different species of plants but also between individual plants of the same species, in which it depends very much on the age of plants, the season, the conditions under which they have been grown and on the temperature or quantity of illumination to which they have been exposed for some time before irradiation (Bawden & Kleczkowski, 1960; Bawden & Sinha, 1961). Consequently the relationship between the amount of irradiation and the effect on capacity of a given leaf to support a given virus is an individual property, and can no more be expressed in generally applicable terms than can the relationship between virus concentration in the inoculum and the number of lesions it will produce. The conditions so far known to increase susceptibility of capacity to UV also increase susceptibility to virus infection, but whether the connection is more than fortuitous has yet to be established.

The total effect of UV radiation on capacity can be determined only by putting leaves in darkness after they are irradiated, for exposure to daylight counteracts the damage (photoreactivation) (Bawden & Kleczkowski, 1952, 1960; Benda, 1955; Bawden & Sinha, 1961). The figure shows the results of two experiments in which *Nicotiana glutinosa* leaves were exposed to different doses of UV radiation immediately before they were inoculated with tobacco mosaic virus, after which half of the leaves were kept for 24 hours in darkness and half were exposed to daylight. The much greater susceptibility to UV of the leaves used in Experiment 2 is obvious, but the effect of photoreactivation is more spectacular in Experiment 1. In both experiments the numbers of lesions formed after photoreactivation depended on the dose of UV irradiation. With

small doses, photoreactivation increased the numbers above that of the non-irradiated controls; with intermediate doses, it restored them to the levels of the controls; with larger doses it increased the numbers but did not restore them to the levels of the controls. The effects of irradiation are obviously complex. Some are reversed by photoreactivation, and some are not. The increase in numbers of lesions above the original level when leaves were exposed to daylight after exposure to small doses of UV radiation may mean that the radiation can increase the leaf's capacity directly, or that photoreactivation can over-compensate the radiation damage and thereby



The figure shows results of two experiments in which halves of leaves of *Nicotiana glutinosa* were irradiated at λ 254 $m\mu$ and then the whole leaves were inoculated with a solution of purified tobacco mosaic virus; L = leaves in daylight after inoculation; D = leaves in darkness for 24 hours after inoculation.

make conditions in some cells such that infection can now occur, although it would not had the cells remained in their original state.

The time required for photoreactivation to be completed after leaves have been given different doses of UV radiation has not been studied in detail, but with moderate doses it probably happens in a few hours in ordinary daylight. The results of inoculating leaves immediately after they have been irradiated differ with different viruses and with different types of inocula. The figure shows that with tobacco mosaic virus considerably more infections are obtained when irradiated leaves are exposed to daylight than when kept in darkness. However, this does not happen with inoculum of free nucleic acid from tobacco mosaic virus, with which no more, or only very slightly more, lesions are obtained on UV-irradiated leaves

exposed to daylight than on those kept in darkness (Bawden & Kleczkowski, 1960). When the irradiated leaves are exposed to daylight for 3 hours before they are inoculated the nucleic acid behaves like the intact virus, and the numbers of lesions formed by the two types of inoculum are equally increased by exposure to daylight. It seems that, whereas the nucleic acid cannot survive infective in irradiated cells until their capacity is restored by photoreactivation, intact particles of the virus can. Different viruses differ in their ability to survive in irradiated cells undergoing photoreactivation. The Rothamsted tobacco necrosis virus inoculated to irradiated French bean leaves behaves similarly to the nucleic acid from tobacco mosaic virus (Bawden & Kleczkowski, 1952), whereas red clover mottle virus is intermediate in its behaviour between tobacco mosaic virus and the tobacco necrosis virus (Bawden & Sinha, 1961). The nucleic acid of this virus also seems more stable *in vivo* than that of tobacco mosaic virus, for when inoculated to leaves immediately after they are irradiated it gives more lesions on leaves kept in the light than in the dark, though the effect of the light is smaller than with inocula of intact virus particles.

Irradiating virus-infected leaves

Arthur & Newell (1929) found that tobacco mosaic virus "could be killed with a short exposure (to UV-radiation) when spread upon the plant leaf surface if irradiated at once. If irradiated the day following inoculation there was no appreciable killing of the virus. It is apparently impossible to inactivate the virus when it has penetrated far into plant tissue, although irradiations were given of sufficient intensity and quality to kill the whole upper surface of plant leaves".

The subject of the effect on viruses of irradiating virus-infected plants remained (to the reviewer's knowledge) untouched for 25 years, until the results of irradiation studies with bacteria infected with bacteriophages (Luria & Latarjet, 1947; Benzer, 1952; Benzer & Jacob, 1953) stimulated further work (Bawden & Harrison, 1955; Siegel & Wildman, 1956). Unfortunately some concepts brought across from the work with bacteriophage were inapplicable to infected leaves and have led to conclusions that further work has shown to be unjustified. The effect of irradiation has been assessed by comparing the numbers of lesions that develop on irradiated halves of leaves with those that develop on unirradiated halves. Differences were attributed solely to the inactivating effect of radiation on virus particles, whereas what was measured was the effect on what can be called "infective centres", and their exact nature is unknown. They may be virus-infected cells or groups of cells, virus particles that are about to infect or cells that are about to be infected. Thus, destroying an infective centre may mean inactivating virus, or affecting cells or virus-cell association.

Effects of irradiating leaves at different times after inoculation on numbers of lesions not only confirmed Arthur & Newell's (1929) conclusion that virus soon spreads from epidermis into deeper tissue where it is protected from the radiation but has also established some other phenomena. Thus, with a tobacco necrosis virus in French bean leaves (Bawden & Harrison, 1955), and with tobacco mosaic

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virus in leaves of *Nicotiana glutinosa* (Siegel & Wildman, 1956), the resistance of infective centres to UV radiation remains unchanged for a time after inoculation, after which it increases steadily and rapidly, until ultimately doses of the radiation much larger than those initially required to prevent lesion formation were ineffective. The reason for the lag period after inoculation before infective centres start to increase their resistance to irradiation (which differs characteristically with different viruses and virus strains) remained without any explanation until Siegel, Ginoso & Wildman (1957) found that it was abolished when plants were inoculated with free nucleic acid isolated from tobacco mosaic virus instead of with the intact virus. This has since been found to be so also with a tobacco necrosis virus (Kassanis, 1960) and with red clover mottle virus (Bawden & Sinha, 1961). The difference between the behaviour of the nucleic acids and intact viruses suggests that the nucleic acids dispense with some early step in the infection process required by intact viruses, and this perhaps provides the strongest evidence for the current idea that a first step in infection normally entails the nucleic acid moiety separating from the protein moiety of the virus.

This idea may be correct, and the results of some other experiments fit readily to it, as, for example, the fact that irradiated nucleic acid from tobacco mosaic virus is photoreactivable immediately it is inoculated to leaves, whereas particles of potato virus X have to wait for 30 minutes or more. However, not all experimental results fit to the idea. For example, as tobacco mosaic virus survives in irradiated leaves through the period while the leaves are photoreactivated and its nucleic acid does not, and as nucleic acid is much more susceptible to UV radiation than the intact virus, if the lag period is the time required for the nucleic acid to become free, irradiating leaves after the period has passed would be expected to inactivate more infective centres than are inactivated immediately after inoculation, but this does not happen (Bawden & Kleczkowski, 1960). This, however, does not disprove the idea, because in the normal process of infection nucleic acid may, after separating from virus protein, immediately combine with some other material, which may increase its stability and resistance to UV radiation. The combination may completely protect the nucleic acid from photoreversible kind of damage by UV, as does the combination with the virus-protein in the original virus particle. Therefore, the fact that infective centres irradiated after the lag period was over could not be photoreactivated to any greater extent than when irradiated earlier (Bawden & Kleczkowski, 1960) also does not disprove the idea of the nucleic acid separating from the virus-protein *in vivo*. Moreover, the effects of UV-irradiation and of photoreactivation on the leaf capacity were so great that they might well have obscured relatively small effects on the nucleic acid if this does become free.

Siegel & Wildman (1956) concluded that, when leaves of *Nicotiana glutinosa* are irradiated within a few hours after inoculating with tobacco mosaic virus, infective centres are destroyed at the rate at which the virus is inactivated when irradiated *in vitro*, and attributed the effect of UV on lesion number solely to inactivation of the virus *in vivo*. This seems wrong, because the extent to which

infective centres are affected by UV even immediately after inoculation depends on the condition of the plant and on whether irradiated leaves are exposed to daylight or kept in darkness, although the virus itself is not photoreactivable after UV-irradiation (Bawden & Kleczkowski, 1960). Among the factors that can affect susceptibility of infective centres to UV is temperature or illumination to which the plant has been exposed for a day or so before irradiation and inoculation, the age of the plant and the season of the year (Bawden & Kleczkowski, 1960; Bawden & Sinha, 1961).

Because the inactivation lines obtained by plotting logs of percentages of lesion survival against doses of irradiation were approximately straight when irradiations were done within a few hours after inoculation with a tobacco necrosis virus or tobacco mosaic virus, Bawden & Harrison (1955) and Siegel & Wildman (1956) concluded that they had disproved the dose hypothesis of infection and established that lesions develop from cells infected by single virus particles. However, as Bawden & Kleczkowski (1960) showed, the results of the irradiation experiments neither prove nor disprove that infections are initiated by single virus particles.

The claims by Bawden & Harrison (1955) and Siegel & Wildman (1956) that changes in the shape of the inactivation lines from previously straight lines to curves of "multiple-hit" type at different times after inoculation show the times when virus particles started multiplying also seem unwarranted, because they neither take into account possible changes in the condition of infected cells, which may alter susceptibility of the cells to UV radiation, nor the fact that to prevent lesion formation larger doses of the radiation are needed some hours after inoculation than immediately after. The larger doses are obviously likely to have more effect on the capacity of cells to support virus multiplication. Moreover, the results obtained by Bawden & Harrison (1955) with a tobacco necrosis virus do not justify the conclusion that the inactivation lines do change some hours after inoculation to a curve of "multiple-hit" type. The curve they drew is not typical of a "multiple-hit" curve, and in drawing this curve the numbers of lesions were transformed according to a dilution curve that related numbers of lesions to virus concentration in the inoculum, whereas the actual numbers should have been used. This transformation enhanced the curving, which is so slight that it seems reasonable to assume that the series of inactivation lines they obtained were all almost straight and differed from each other only in their slopes. The inactivation lines published by Siegel & Wildman (1956) for tobacco mosaic virus in leaves of *Nicotiana glutinosa* do change from straight lines to curves of "multiple-hit" type, but how to interpret this is uncertain. If the change does reflect the fact that infected cells now contain more than one virus particle, then it seems that a comparable stage is not detectable in French bean leaves infected with a tobacco necrosis virus, and a "multiple-hit" curve is not typical of all virus-host combinations.

The irradiation experiments with virus-infected leaves have revealed a number of phenomena. Whether further irradiation experiments alone can explain these phenomena, however, is doubtful. At the moment irradiation does provide a method of detecting

changes in infected cells that otherwise would remain undetectable, but other methods of study will probably be needed to show the nature of these changes.

REFERENCES

- ARTHUR, J. M. & NEWELL, J. M. (1929). The killing of plant tissue and the inactivation of tobacco mosaic virus by ultraviolet radiation. *Amer. J. Bot.* **16**, 338-353.
- BAWDEN, F. C. & HARRISON, B. D. (1955). Studies on the multiplication of a tobacco necrosis virus in inoculated leaves of French bean plants. *J. gen. Microbiol.* **13**, 494-508.
- BAWDEN, F. C. & KLECZKOWSKI, A. (1952). Ultra-violet injury to higher plants counteracted by visible light. *Nature, Lond.* **169**, 90.
- BAWDEN, F. C. & KLECZKOWSKI, A. (1953). The behaviour of some plant viruses after exposure to ultraviolet radiation. *J. gen. Microbiol.* **8**, 145-156.
- BAWDEN, F. C. & KLECZKOWSKI, A. (1955). Studies on the ability of light to counteract inactivating action of ultraviolet radiation on plant viruses. *J. gen. Microbiol.* **13**, 370-382.
- BAWDEN, F. C. & KLECZKOWSKI, A. (1959). Photoreactivation of nucleic acid from tobacco mosaic virus. *Nature, Lond.* **183**, 503-504.
- BAWDEN, F. C. & KLECZKOWSKI, A. (1960). Some effects of ultra-violet radiation on the infection of *Nicotiana glutinosa* leaves by tobacco mosaic virus. *Virology*, **10**, 163-181.
- BAWDEN, F. C. & PIRIE, N. W. (1938a). Liquid crystalline preparations of potato virus X. *Brit. J. exp. Path.* **19**, 66-82.
- BAWDEN, F. C. & PIRIE, N. W. (1938b). Crystalline preparations of tomato bushy stunt virus. *Brit. J. exp. Path.* **19**, 251-263.
- BAWDEN, F. C. & SINHA, R. C. (1961). Effects of ultraviolet radiation on infection by intact and phenol-disrupted red clover mottle virus. *Virology* (in the press).
- BENDA, G. T. A. (1955). Some effects of ultra-violet radiation on leaves of French bean (*Phaseolus vulgaris* L.). *Ann. appl. Biol.* **43**, 71-85.
- BENZER, S. (1952). Resistance to ultra-violet light as an index to the reproduction of bacteriophage. *J. Bact.* **63**, 59-72.
- BENZER, S. & JACOB, F. (1953). Etude de développement du bactériophage au moyen d'irradiation par la lumière ultraviolette. *Ann. Inst. Pasteur*, **84**, 186-204.
- CHESSIN, M. (1958). Light quality and photoreactivation of plants and viruses. *Ann. appl. Biol.* **46**, 388-392.
- DUGGAR, B. M. & HOLLAENDER, A. (1934a). Irradiation of plant viruses and of microorganisms with monochromatic light. I. The virus of typical tobacco mosaic and *Serratia marcescens* as influenced by ultraviolet and visible light. *J. Bact.* **27**, 219-239.
- DUGGAR, B. M. & HOLLAENDER, A. (1934b). Irradiation of plant viruses and of microorganisms with monochromatic light. II. Resistance to ultra-violet radiation of a plant virus as contrasted with vegetative and spore stages of certain bacteria. *J. Bact.* **27**, 241-256.
- DULBECCO, R. (1950). Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. *J. Bact.* **59**, 329-347.
- HARRISON, B. D. & NIXON, H. L. (1959). Some properties of ineffective preparations made by disrupting tobacco rattle virus with phenol. *J. gen. Microbiol.* **21**, 591-599.
- HOLLAENDER, A. & DUGGAR, B. M. (1936). Irradiation of plant viruses and of microorganisms with monochromatic light. III. Resistance of the virus of typical tobacco mosaic and *Escherichia coli* to radiations from λ 3000 to λ 2250 Å. *Proc. nat. Acad. Sci. Wash.* **22**, 19-24.
- KASSANIS, B. (1960). Comparison of the early stages of infection by intact and phenol-disrupted tobacco necrosis virus. *Virology*, **10**, 353-369.
- KELNER, A. (1949). Effect of visible light on the recovery of *Streptomyces griseus* conidia from ultraviolet radiation injury. *Proc. nat. Acad. Sci. Wash.* **35**, 73-79.
- KELNER, A. (1951). Action spectra for photoreactivation of ultraviolet-irradiated *Escherichia coli* and *Streptomyces griseus*. *J. gen. Physiol.* **34**, 835-852.

- KLECZKOWSKI, A. (1954). Stability of chymotrypsin and tobacco mosaic virus decreased by ultraviolet radiation. *Biochem. J.* **56**, 345-349.
- KLECZKOWSKI, A. (1960). Effects of nonionizing radiation on plant viruses. *Ann. N.Y. Acad. Sci.* **83**, 661-669.
- LEA, D. E. & SMITH, K. M. (1940). The inactivation of plant viruses by radiation. *Parasitology*, **32**, 405-416.
- LENNOX, E. S., LURIA, S. E. & BENZER, S. (1954). On the mechanism of photoreactivation of ultraviolet-inactivated bacteriophage. *Biochim. biophys. Acta*, **15**, 471-474.
- LURIA, S. E. & LATARJET, R. (1947). Ultraviolet irradiation of bacteriophage during intracellular growth. *J. Bact.* **53**, 149-163.
- MAQUENNE, L. & DEMOUSSY (1909). Influence de rayons ultra-violet sur la végétation des plantes vertes. *C.R. Acad. Sci. Paris*, **149**, 756-760.
- MCLAREN, A. D. & TAKAHASHI, W. N. (1957). Inactivation of infectious nucleic acid from tobacco mosaic virus by ultraviolet light (2537 Å). *Radiation Res.* **6**, 532-542.
- MULVANIA, M. (1926). Studies on the nature of the virus of tobacco mosaic. *Phytopathology*, **16**, 853-871.
- OSTER, G. & MCLAREN, A. D. (1950). The ultraviolet light and photosensitized inactivation of tobacco mosaic virus. *J. gen. Physiol.* **33**, 215-228.
- PRICE, W. C. & GOWEN, J. W. (1937). Quantitative studies of tobacco mosaic virus inactivation by ultra-violet light. *Phytopathology*, **27**, 267-282.
- RUSHIZKY, G. W., KNIGHT, C. A. & MCLAREN, A. D. (1960). A comparison of the ultraviolet-light inactivation of infectious ribonucleic acid preparations from tobacco mosaic virus with those of the native and reconstituted virus. *Virology*, **12**, 32-47.
- SHUGAR, D. & WIERZCHOWSKI, K. L. (1958). Photochemistry of nucleic acids, nucleic acid derivatives and related compounds. *Postępy Biochem., Polska Akad. Nauk*, **4**, 243-296.
- SIEGEL, A., GINOZA, W. & WILDMAN, S. G. (1957). The early events of infection with tobacco mosaic virus nucleic acid. *Virology*, **3**, 554-559.
- SIEGEL, A. & NORMAN, A. (1958). Action spectra for two strains of tobacco mosaic virus. *Virology*, **6**, 725-731.
- SIEGEL, A. & WILDMAN, S. G. (1956). The inactivation of the infectious centers of tobacco mosaic virus by ultraviolet light. *Virology*, **2**, 69-82.
- SIEGEL, A., WILDMAN, S. G. & GINOZA, W. (1956). Sensitivity to ultraviolet light of infectious tobacco mosaic virus nucleic acid. *Nature, Lond.* **178**, 1117-1118.
- SMITH, F. F. (1926). Some cytological and physiological studies of mosaic diseases and leaf variegations. *Ann. Mo. bot. Gdn*, **13**, 425-484.
- STANLEY, W. M. (1936). The inactivation of crystalline tobacco mosaic virus. *Science*, **83**, 626-627.
- TANADA, T. & HENDRICKS, S. B. (1953). Photoreversal of ultraviolet effects in soybean leaves. *Amer. J. Bot.* **40**, 634-637.
- ZELLE, M. R. (1955). Effect of radiations on bacteria. In *Radiation Biology*, edited by A. Hollaender. New York, McGraw-Hill Book Co. Inc. Vol. 2, pp. 365-430.