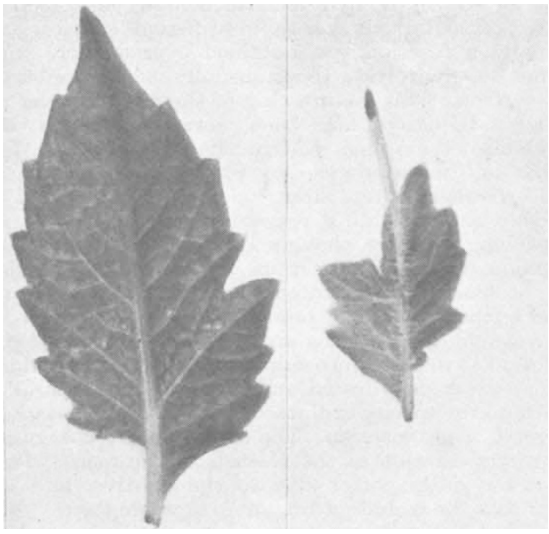

Fig. 1. Leaves of dahlia variety Jean showing enations

to a strain of cucumber mosaic virus[6]. However, experiments have shown that these enations in *Dahlia variabilis* var. Jean were induced not by a virus complex but by a strain of tomato spotted wilt virus. When leaf extracts from the diseased plant were inoculated into a range of test plants, this virus was identified, but no other was isolated; no virus transmission to the same test plants resulted when leaf extracts were maintained at 43° C. for 10 min. before inoculation. Apterous females of the aphid *Myzus persicae* Sulz., given varying acquisition and test-feeding times, failed to transmit any virus from the diseased plant to young healthy dahlia seedlings, although in comparable experiments this species readily transmitted dahlia mosaic virus and cucumber mosaic virus from infected to healthy dahlias. When scions from the diseased plant were grafted on to the indicator variety, Willy Den Ouden, symptoms attributable to tomato spotted wilt virus were observed; when grafted to healthy plants of the same variety enations were rarely observed.

This is thought to be the first report of tomato spotted wilt virus inducing enation formation in any species of its extensive host-range, although strains of the virus have been intensively studied in relation to the pathology of tomato, tobacco and other crops.

There can be little doubt that oak-leaf, ringspot and yellow ringspot symptoms observed on dahlias in Britain and shown by these and other experiments to be induced by tomato spotted wilt virus are identical with those originally reported to be caused by separate non-sap-transmissible viruses in America[4]. Consequently, the terms dahlia oak-leaf virus[4], dahlia ringspot virus[4] and dahlia yellow ringspot virus[4] are considered to be invalid, and it is suggested that their use be discontinued.

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[1] Jensen, J. H., *Contr. Boyce Thomp. Inst.*, **5**, 129 (1933).
[2] Govier, D. A., *Ann. App. Biol.*, **45**, 62 (1957).
[3] Smith, K. M., *Ann. App. Biol.*, **19**, 305 (1932).
[4] Brierley, P., *Contr. Boyce Thompson Inst.*, **5**, 235 (1933).
[5] Brierley, P., *Plant Dis. Rep.*, **34**, 363 (1950).
[6] Grainger, J., "The Improvement of Dahlia Stocks" (Pub. British Dahlia Growers' Assoc., 1953).

PATHOLOGY

Effect of Neutral Red on Plaque Formation by Fowl Plague Virus

In a previous paper on the formation of plaques by fowl plague virus in Petri dishes of chick embryo cells, it was briefly reported that neutral red had the effect of reducing the number and size of the plaques formed by the virus if added to the cells at the beginning of an assay experiment, but a much smaller effect if added after three days[1].

A similar effect has been observed with poliomyelitis and western equine encephalitis viruses[2], and also with vesicular stomatitis virus[3].

A more quantitative study of this phenomenon suggests that the relation between the quantity of neutral red and the cell numbers is critical (Table 1), and also that time of reduction in plaque numbers is proportional to the concentration of neutral red (Table 2). The Rostock strain of fowl plague virus and the agar cell suspension plaque assay technique of Cooper[4] were used in all the experiments, as described in the earlier communication. Neutral red (Gurr) was used, and a standard volume was incorporated in the agar to make the final concentration in each Petri dish 1 : 10,000.

Table 1. Effect of Cell Numbers

Cells per Petri dish	Neutral red	Appearance on fourth day	Mean plaque count	Inhibition (per cent)
6×10^7	—	Plaques	71	—
6×10^7	+	Very small plaques	32	55
3×10^7	+	Toxic effect, with peripheral pallor. No plaques seen	—	—

Table 2. Effect of Varying Quantity of Neutral Red

Experiment No.	Quantity of neutral red	Mean plaque count	Reduction of plaque count (per cent)
1	Nil	61	—
	Standard	28	55
2	Nil	70	—
	Half-standard	51	27

The effect of neutral red appears early; that is, the reduction in plaque count is only slight if applied later, for example, on the third day. In this experiment (Table 3), no stain was added at the beginning, but six Petri dishes were stained on the third day and six on the fourth. All were read on the fourth day:

Table 3

Stained	Mean plaque count
Third day	22
Fourth day	26

In the experiments of Darnell *et al.*[2], the classical cell sheet technique was used, and they give reason for supposing that the neutral red acts on the infected cell, as adsorption of further virus is prevented by addition of agar overlay.

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[1] Waterson, A. P., *Arch. ges. Virusforsch.*, **8**, 113 (1958).
[2] Darnell, J. E., Lockhart, R. Z., and Sawyer, T. K., *Virology*, **6**, 567 (1958).
[3] McClain, M. E., and Hackett, A. J., *J. Immunol.*, **80**, 356 (1958).
[4] Cooper, P. D., *Virology*, **1**, 397 (1955).