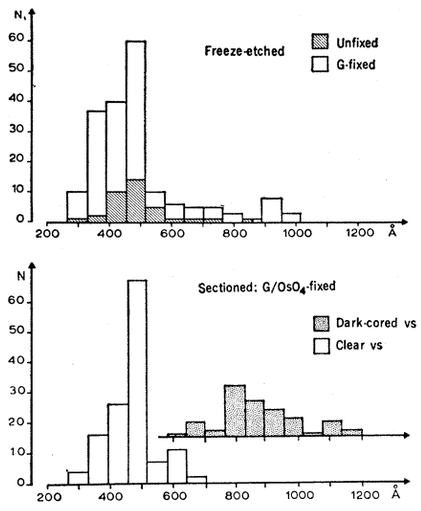

Fig. 3. Histograms of diameters of freeze-etched and conventionally prepared synaptic vesicles. (Top) Histograms of freeze-etched fixed and unfixed tissue. Note that peaks and ranges correspond closely. However, clear and dark-cored vesicles (*vs*) could not be separated. Histogram of conventionally treated vesicles (see bottom) suggests that vesicles exceeding 800 Å are dark-cored. (Bottom) Histogram of synaptic vesicles in whole tissue sections fixed in glutaraldehyde and OsO_4 (conventional electron micrographs). Note that the peak coincides with that of freeze-etched vesicles (top). The ranges of clear and dark-cored vesicles overlap slightly.

tions (Fig. 3); even larger ones may be seen occasionally. On the other hand, the diameters of clear vesicle profiles extend up to 700 Å. Thus, there is an overlap in size between the two categories of vesicles. For this reason, it may be concluded that in freeze-etched preparations only vesicles exceeding diameters of 800 Å may safely be classified as being dark-cored.

Plasmalemmal stomata that were described in nonsynaptic regions of nerve terminals in a previous communication (*4*) are confirmed in the present study (Fig. 1). These stomata have a diameter of approximately 350 Å and are interpreted as pinocytotic vesicles. Their relevance to the problem of formation of synaptic vesicles cannot be clarified with morphological studies alone.

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20 March 1969

Aphid Transmission of Tobacco Mosaic Virus

Abstract. *Aphids* (Myzus persicae *Sulz.) can acquire tobacco mosaic virus from tobacco leaves coated with a virus suspension and inoculate it into healthy leaves. Transmission depends on virus concentration, period of acquisition, previous feeding history of the aphids, and time between acquisition and transmission feedings. Aphids whose stylets are cut do not transmit the virus.*

Aphids transmit plant viruses by carrying them either internally (circulative viruses) or at their stylets (stylet-borne viruses). However, several viruses are not transmitted by aphids. Despite various attempts, no conclusive evidence has been presented why tobacco mosaic virus (TMV) cannot be transmitted by aphids, although as the result of this research some aspects of the relation between aphids and TMV could be clarified (*1–3*). For example, ingestion of TMV by aphids (*1*) and its subsequent discharge into a feeding substrate was demonstrated (*3, 4*). *Myzus persicae* Sulz. and *M. circumflexus* (Buckton) were shown to inoculate TMV when probing on leaves previously sprayed with TMV (*5*). We have found that *M. persicae* can acquire TMV from virus-covered leaves and inoculate it into healthy leaves.

Green peach aphids *M. persicae* were reared on Chinese cabbage (*Brassica pekinensis* Rupr.) or tobacco (*Nicotiana tabacum* L.). *Nicotiana glutinosa* L. was used as a test plant since it produces local lesions upon inoculation with TMV and is a more sensitive indicator when inoculated by aphids than is *N. tabacum* var. Xanthi-nc (*6*).

Leaves of *N. glutinosa* were detached, immersed in or sprayed with a suspension of purified or unpurified virus, and placed on plastic support rings in petri dishes partially filled with water. As soon as the virus suspension had dried, about 30 aphids were placed on the leaves for 1 to 3 hours. Test leaves were then cut in half; one-half was used for transmission, the other as control. Aphids were collected from virus-covered leaves into a plastic container and carefully

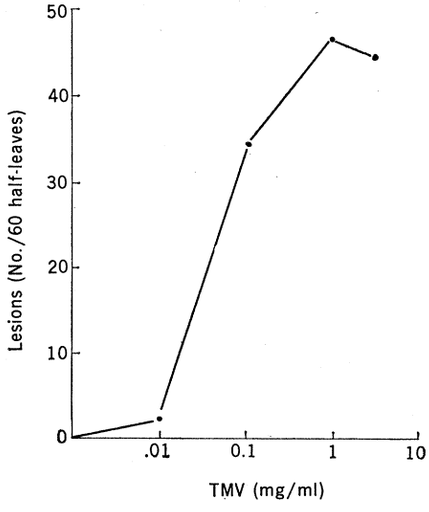

Fig. 1. Effect of virus concentration on transmission expressed as number of lesions produced by aphids that had previously probed on tobacco leaves covered with various concentrations of tobacco mosaic virus. Thirty aphids were placed on each half-leaf.

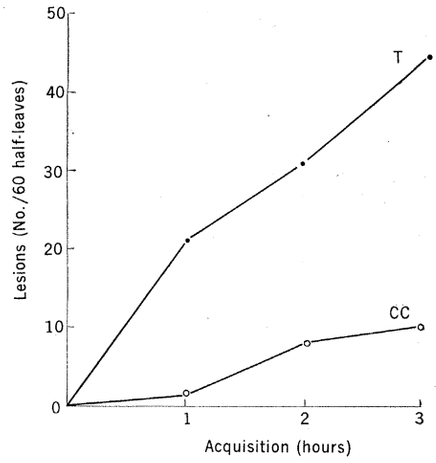

Fig. 2. Effect of length of acquisition period on transmission expressed as number of lesions produced by aphids that had previously probed on tobacco leaves covered with purified tobacco mosaic virus (5 mg/ml); *T*, aphids reared on tobacco; *CC*, aphids reared on Chinese cabbage. Thirty aphids were placed on each half-leaf.

dropped in the center of a test half-leaf. Half-leaves were placed on rings as described above. After 4 hours on the test leaves, aphids were killed by saturating the atmosphere in the petri dish with Thiodane. Lesions were counted after 3 days, and initially all lesions were indexed to see whether they were caused by TMV.

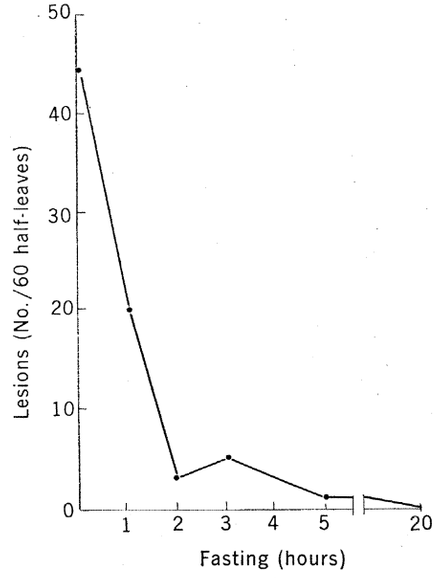

Fig. 3. Effect of fasting on virus transmission expressed as number of lesions produced by aphids that were starved for various periods after having stayed for 3 hours on tobacco leaves covered with purified tobacco mosaic virus (5 mg/ml). Thirty aphids were placed on each half-leaf.

For studying retention of TMV, aphids were left for 3 hours on leaves that were sprayed with purified TMV (5 mg/ml). They were placed in plastic bottles, starved for various periods, and then placed on healthy leaves as described.

Two controls were used. First, for each half-leaf receiving viruliferous aphids, another half-leaf was treated similarly except for receiving no aphids. This should monitor the level of background contamination inherent in the system. Second, 1200 aphids that had stayed on virus-covered (5 mg/ml) leaves for 3 hours were anesthetized, and their stylets were cut off; the insects were then transferred to test leaves. This should indicate whether aphids carry TMV on their tarsi and inoculate TMV by walking over the leaf surface.

We showed that *M. persicae* can acquire TMV from virus-covered leaves, carry it to healthy leaves, and inoculate the virus. Lesions on *N. glutinosa* developed randomly over the leaf surface and only rarely in areas where aphids had been dropped. Transmission was noticeable after aphids had probed on leaves covered with 0.01 mg of purified TMV per milliliter, and it increased in efficiency up to 1 mg/ml (Fig. 1). Aphids could also acquire TMV from leaves covered with crude extracts (frozen infected tissue extracted without buffer in an Erweka juice extractor) producing an average of 16 lesions per 60 leaves. Efficiency of transmission depended on period of acquisition and previous feeding history of the aphids (Fig. 2). Aphids from *Brassica pekinensis* were less efficient vectors than aphids reared on tobacco; they were more restless, and began to drop off after 4 to 5 hours on tobacco leaves. Aphids could retain TMV for several hours after they had left the leaves, but transmission decreased after 2 to 3 hours of fasting (Fig. 3). No lesions developed in the controls. Aphids whose stylets had been cut off were less mobile than normal aphids, but their mobility increased greatly after application of insecticide.

Previous work has shown that aphids can acquire and release TMV, but no transmission was demonstrated (*3*, *4*). Natural transmission of TMV has been claimed, but several of these claims could not be substantiated (*1*, *2*).

We conclude that the transmission we obtained did result from the probing or feeding of aphids on the test leaves (or both) because aphids without stylets did not produce lesions, and the transmission was less efficient with aphids not adapted to tobacco.

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Irreversible Inhibition of Nuclear Exoribonuclease by Thymidine-3′-Fluorophosphate and p-Haloacetamidophenyl Nucleotides

Abstract. *Exoribonuclease purified from Ehrlich ascites tumor cell nuclei and in intact HeLa cell nuclei is irreversibly inactivated by low concentrations of* p-*bromo- and* p-*iodoacetamidophenyl nucleotides and by thymidine-3′-fluorophosphate. Iodoacetate, bromoacetate, and thymidine-5′-fluorophosphate do not affect the enzyme. Although* p-*haloacetamidophenyl nucleotides inactivate ribonucleic acid polymerase of isolated HeLa cell nuclei, thymidine-3′-fluorophosphate does not affect the activity of this enzyme in vitro.*

Inhibitors that form covalent bonds with enzymes at their catalytic or regulatory active sites (active-site-directed irreversible enzyme inhibitors) are important tools for investigating the structure and function of many enzymes (*1*). In conjunction with studies on the cellular function and molecular mechanism of action of a nuclear exoribonuclease that selectively destroys single-stranded or rapidly labeled RNA (*2*), we have synthesized a new series of *p*HAP (*3*) nucleotides which irreversibly inhibit this enzyme. The aim of these studies is to develop new agents which will be specific and potent