ON THE MECHANISM OF TRANSMISSION OF NON-PERSISTENT PHYTOPATHOGENIC VIRUSES BY APHIDS

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Summary

The following is a working hypothesis, based on published data, to elucidate the mechanism of transmission of non-persistent viruses by aphids: The stylets of the vector become contaminated with virus during probing in infected

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tissues. Only this virus is of significance in disease transmission, and ingested virus plays no part in the process. Some of the virus on the stylets is inactivated by salivary fluids. Viruses differ in their susceptibility to this inhibition and different species of aphids vary in the production of the inhibitor. Fasting decreases the activity of the salivary inhibitors.

Experimental evidence has been sought to determine whether virus is carried on the stylets, and whether salivary secretions are inhibitory. Although direct proof of these points is lacking, there is evidence that infective virus is carried mechanically, and that some viruses are sensitive to components of the saliva of certain insects. A study of the effects of fasting on the physiology of aphid vectors has shown that fasting has no effect on digestive enzyme formation, the rate of penetration of the stylets into the leaf, or the amount of plant material ingested. Fasting does have an effect on aphid feeding behaviour and on the production of saliva. Finally, explanations for vector specificity and differences in vector efficiency are examined.

It is concluded that there is evidence in favour of the working hypothesis.

I. Introduction

The majority of viruses causing diseases of plants are transmitted by insects. These insect-transmitted, phytopathogenic viruses fall into two main groups, designated as "persistent" and "non-persistent" viruses by Watson and Roberts (1939). The vectors of persistent viruses remain infective for long periods, and there is usually a latent period following an acquisition feed during which the virus cannot be transmitted. All the leafhopper-borne viruses and some aphid-borne viruses (e.g. potato leaf roll virus, pea enation mosaic virus, filaree red leaf virus, tobacco rosette virus, a strawberry crinkle virus, and others) belong to this category. The vectors of non-persistent viruses can transmit immediately after an infective feed, but generally remain infective for only a short period of time. This group includes the majority of aphid-borne viruses, many of which are of considerable economic importance.

The mechanism of transmission of non-persistent viruses is uncertain; Black (1954) has recently said that "our understanding of what actually occurs during aphid transmission is far from complete." This paper presents a hypothesis that appears to explain most of the data available, and gives details of experiments to test the validity of some aspects of the hypothesis.

Four mechanisms of aphid transmission of non-persistent viruses have previously been suggested. These may be termed (*a*) biological transmission (see, for example, Smith and Lea 1946), (*b*) transmission by the salivary apparatus (Bradley 1952), (*c*) transmission by regurgitation, and (*d*) mechanical transmission (Hoggan 1931, 1933, 1934). The route taken by the virus in each of these hypothetical mechanisms is illustrated in Figure 1.

There are objections to each of these suggestions, and consideration of published data on aphid transmission of non-persistent viruses led to the following working hypothesis:

The stylets of the aphid become contaminated with virus during probing in infected tissue. Only this fraction of the virus is of significance in disease transmission; some of the virus may be ingested, but upon reaching the midgut it plays no part in disease transmission. Some of the virus on the stylets is

inactivated by the salivary fluids. This inactivation requires time, and after short periods sufficient still remains for active virus to be introduced into a susceptible host plant and to initiate infection during the probing by the insects' stylets. Some viruses are very sensitive to inactivation by the saliva, and once a salivary sheath is formed transmission of the diseases caused by these sensi-

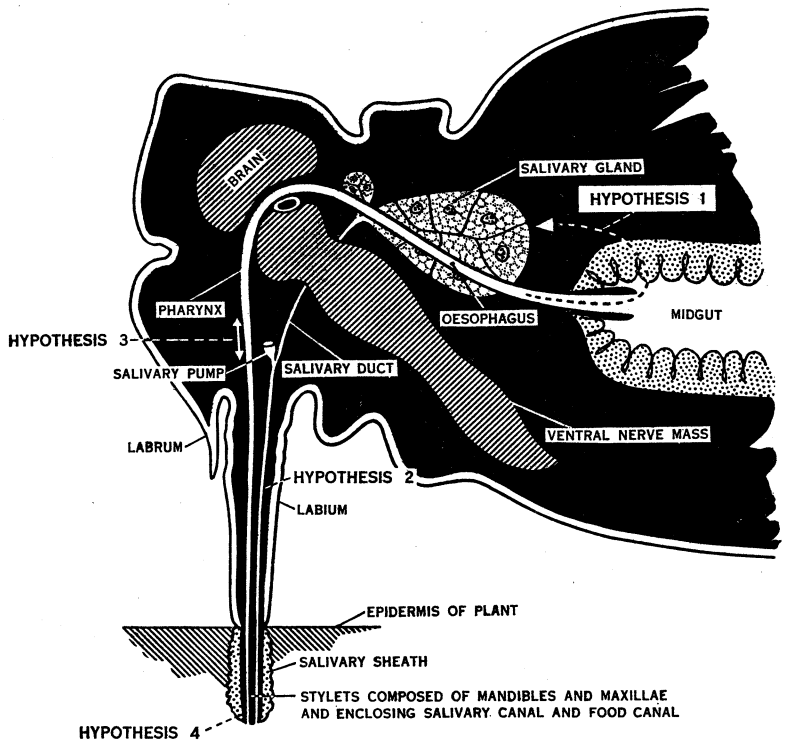

Fig. 1.—Diagram of median longitudinal section of anterior part of an aphid, showing the supposed course of virus in each of the four hypotheses of transmission. In Hypothesis I (biological transmission) the virus is thought to be ingested, to pass to the midgut where absorption occurs; the virus then reaches the haemocoele, passes to the salivary glands, and is transmitted to a susceptible host in the saliva. In Hypothesis II (transmission by the salivary apparatus) the virus is thought to contaminate the salivary canal and to be forced out at the commencement of the subsequent feeding period. In Hypothesis III (transmission by regurgitation) the virus is thought to be ingested and regurgitated during a subsequent feed. In Hypothesis IV (mechanical transmission) the virus is thought to contaminate the stylets.

tive viruses is no longer possible through that feeding puncture. The insect produces the salivary inhibitor less readily after a period of fasting than it does if removed from one host directly to another. Furthermore, viruses differ in their susceptibility to the salivary inhibitors from different vectors.

Although it is basically not new, this "modified mechanical" hypothesis stresses the mechanical role of aphid vectors, towards which much criticism had been directed; but much of this criticism is vitiated by the emphasis on the inhibitory role of the saliva of the aphid.

II. Experimental Tests of the Validity of Some Aspects of the Hypothesis

Details of experiments to test some aspects of the modified mechanical hypothesis are presented in this section under the following headings:

(*a*) Is virus carried on the mouth-parts?
(*b*) Are viruses inhibited by insect salivary secretions?
(*c*) What are the effects of fasting upon the aphid vectors?
(*d*) What explanations are available for the observed specificity in virus-vector relationships?

A few negative results are mentioned to indicate certain approaches that have proved unprofitable. Data from the literature are used to corroborate the argument.

Table 1

INSTANCES OF SHORT FEEDING PERIODS THAT RESULTED IN TRANSMISSION OF APHID-BORNE VIRUSES

Thirty sec arbitrarily allowed for transference from infected plant to initiation of feeding on uninfected plant

Virus	Vector	Duration (sec) of		Aproximate Total Elapsed Time
		Acquisition Feed	Inoculation Feed	
Cauliflower mosaic	*M. persicae*	23	30	1 Min 23 sec
Cauliflower mosaic	*M. persicae*	60	22	1 Min 52 sec
Cauliflower mosaic	*B. brassicae*	60	25	1 Min 55 sec
Cauliflower mosaic	*B. brassicae*	60	60	2 Min 30 sec
Potato virus Y	*M. persicae*	28	20	1 Min 18 sec
Potato virus Y	*M. persicae*	50	15	1 Min 35 sec

(*a*) *Is Virus Carried on the Mouth-parts?*

(i) *Duration of the Transmission Cycle.*—Several authors (e.g. Sylvester 1949*b*, 1950*b*, 1952; Bradley 1952; Hamlyn 1953) have shown that the total time between the initiation of an acquisition feed and the completion of a successful inoculation feed may be no more than 1-2 min. We have confirmed this with *Myzus persicae* (Sulz.) and *Brevicoryne brassicae* (L.) transmitting cauliflower mosaic,* and *M. persicae* transmitting potato Y virus† (Table 1).

Experiments on feeding of *M. persicae* (Day and Irzykiewicz 1953) and observations on the stylets of this aphid during probing and feeding indicate that it is unlikely that sufficient material to reach the midgut could be ingested

* Cauliflower mosaic virus used in this paper was obtained from an infected cauliflower in Canberra. We are indebted to Mr. L. L. Stubbs, Victorian Department of Agriculture, for typing this virus.

† Potato Y virus was obtained through the courtesy of Dr. E. M. Hutton, Division of Plant Industry, C.S.I.R.O.

during the very short "feeds" that resulted in virus transmission. The conclusion seems inescapable that the infective virus is carried on the mouth-parts or in the anterior part of the foregut.

From the viewpoint of the modified mechanical hypothesis it is immaterial whether the virus is carried externally on the stylets or is confined to the salivary or feeding ducts as suggested by Bradley (1952). However, the salivary canal of *M. persicae* cannot have a volume much in excess of 6×10^{-11} ml (diameter approximately 1 μ, length 70 μ); on the other hand, Day and Irzykiewicz (1953) have shown that many times this volume of solution can be carried on the mouth-parts of this aphid.

(ii) *Retention of Virus Through the Moult.*—The stylets of an aphid are shed at each moult and new stylets are produced from within the head capsule. If a virus is carried on the stylets, then it could not be transmitted following a moult. This should therefore provide a test of whether virus is carried on the mouth-parts. Sylvester (1949*a*) reported examples, using *M. persicae* and beet yellow net virus, in which the virus was thought to be retained by the vector from one instar to the next. Attempts to repeat this observation with cauliflower mosaic and *M. persicae* have been uniformly negative. The experiment was performed by maintaining a culture of *M. persicae* on turnip (var. Flat Express) showing severe symptoms of cauliflower mosaic virus. Periodically the plant was searched for aphids in the process of moulting. This process occupied about 20 min and the insects were able to feed within a few minutes of casting the skin. It was imperative that specimens be removed before they had fed and that they be handled with the utmost care. Twenty newly moulted aphids were removed to turnip (var. Flat Express) indicator plants on which feeding was noted. None of these plants became infected, whereas three plants became infected when five non-moulting aphids from the same viruliferous plant were transferred to each of five test plants. From this experiment it is concluded that it is unlikely that cauliflower mosaic virus is retained by its aphid vector after the latter moults. If the modified mechanical hypothesis is correct the ability of an aphid to retain infectivity after a moult would be sufficient to exclude that virus from the category of those transmitted by the modified mechanical mechanism.

(iii) *Experiments Involving Wetting the Stylets with Virus in Solution.*—Bradley (1952) performed an experiment in which he dipped the stylets of aphids into a concentrated solution of tobacco mosaic virus, and then permitted the insects to feed upon the test plants. The absence of infection was taken as evidence that the virus was not carried upon the outside of the insects' stylets.

An attempt was made to compare the ability of *M. persicae* to transmit cucumber mosaic virus* after feeding on an infected leaf or on a concentrated solution of plant juice. Transmission after feeding on the leaf of infected spinach was readily obtained (5 out of 8). Aphids fed through a plastic membrane on sap from a similar leaf produced no infections, but this may have been due to the presence of an inhibitor in the plant (Sill and Walker 1952). Sap

* Obtained through the cooperation of Mr. L. L. Stubbs (see Stubbs 1952*b*).

from infected *Nicotiana glutinosa* L. leaves was therefore clarified by low-speed centrifugation. This solution was highly infectious when mechanically inoculated on to *N. glutinosa* or spinach, but 90 *M. persicae* did not become infectious when fed through a plastic membrane on the solution for from 30 sec to 5 min and then transferred in groups to 14 spinach seedlings. It is therefore likely that the aphid is unable to pick up virus from solution as it does from the leaf. It is evident that the conclusion that the virus is not carried on the exterior of the stylets is not warranted by the results of Bradley's experiments.

(iv) *Conclusions.*—The conclusion that the viruses are carried on the mouth-parts is thus supported by the following: (*a*) the short time between the beginning of the acquisition feed and the end of a successful inoculation feed, together with the evidence that food is not taken into the midgut during the short punctures that suffice for transmission; (*b*) the observation that the virus is not transmissible after the vector moults unless it has further access to the viruliferous host; (*c*) the comparison between the mechanism of aphid-borne viruses and behaviour of certain viruses on the mouth-parts of mosquitoes (see Day and Fenner 1953). It is pointed out that the results of experiments involving wetting the stylets with virus, or on needle transmission, do not oppose the theory that the viruses are carried on the aphids' mouth-parts.

(*b*) *Are Viruses Inhibited by Insect Salivary Secretions?*

(i) *Ingestion of Mosaic Viruses by Aphids.*—Sukhov (1944) concluded that the aphid salivary sheath is responsible for rendering the aphid incapable of transmitting tobacco mosaic virus. He was unable to detect the presence of virus in *M. persicae* feeding on virus-infected plants and concluded, on this slender evidence, that the salivary sheath acts as a filter that absorbs the virus. It has been shown that aphids contain substances that are highly inactivating against tobacco mosaic virus (Black 1939; Smith 1941), and Sukhov's inability to detect virus in the insect does not prove that the salivary sheath functions as a filter to prevent the ingestion of the virus. In fact, we have observed instances of aphids feeding through plastic membranes when the stylets protruded beyond the tip of the salivary sheath. That aphids can, in fact, ingest certain viruses was demonstrated by Severin and Tompkins (1948).

We have made a number of attempts to demonstrate that *M. persicae* ingests tobacco mosaic virus (TMV) in order to refute more directly Sukhov's suggestion. It has proved very difficult to feed aphids on plants containing a high concentration of TMV. However, it has been shown that *M. persicae, B. brassicae,* and *Macrosiphum euphorbiae* (Thom.) ingest TMV from solution although they produce typical salivary sheaths in the liquid. The difficulty of demonstrating the ingestion of virus even from solutions containing high concentrations of TMV clearly shows the reasons for the failure of many previous attempts. Ingestion of virus was finally demonstrated by feeding apterae of the three species for 18 hr on a concentrated TMV solution containing 5 per cent. sucrose presented through a plastic membrane. Care was taken to eliminate

contamination and controls demonstrated that none occurred. The insects were then macerated in a minimum of M/15 phosphate buffer at pH 7.0. Inoculation of *N. glutinosa* with the supernatant following low-speed centrifugation caused no local lesions. However, virus could be separated from the solution by centrifuging in a Spinco ultracentrifuge for 45 min at 107,000g. The pellet was then redissolved in buffer and the solution ultracentrifuged again. The resulting pellet was taken up in buffer and the solution used to inoculate leaves of *N. glutinosa.* Local lesions of TMV were then produced, showing that the inhibiting substances from the aphids had been separated from the virus (cf. Black 1939).

In spite of the demonstration that the salivary sheath does not filter out virus particles, the modified mechanical hypothesis suggests that the saliva of the aphid is responsible for the inability of the insect to transmit the virus. Trypsin is an inhibitor of those mosaic viruses with which it has been tested, but no mechanism has been suggested which allows the insects' proteinase to come in contact with the virus; however, the virus must come into contact with the saliva of an aphid that feeds for more than a few minutes.

Although a method has recently been described for obtaining the saliva of leafhoppers (Braun and Maramorosch 1951), the minute amount produced by aphids makes it unlikely that sufficient could be collected to examine its inhibitory properties directly. An experiment was performed to determine whether the salivary sheath might contain a highly active, diffusable inhibitor. *M. persicae* in groups of 30 were permitted to feed through a plastic membrane for 6 hr upon drops of tobacco mosaic virus in phosphate buffer diluted to 10^{-4}, after which the solution was observed to contain a number of feeding tracks. The virus solution was then inoculated on to half leaves of *N. glutinosa,* the other half leaf being inoculated with a solution of virus that had remained on the plastic membranes without insects feeding on it. No differences were observed between the groups of lesions produced and it was concluded that, even though *M. persicae* is an inefficient vector of tobacco mosaic virus (Hoggan 1934), the supposed inhibitor of aphid saliva was insufficiently active to affect so large a volume of virus.

(ii) *Inhibition by Saliva from* Periplaneta.—It is fully realized that results obtained with insects unrelated to aphids are at best merely indications, but it seemed that the only approach to the problem of salivary inhibition lay in the study of insect species from which sufficient saliva could be collected to perform reproducible experiments. Saliva of the cockroach *Periplaneta americana* (L.) may frequently be obtained as droplets on the mouth-parts (Day 1951). A mixture of virus solution and cockroach saliva was used to inoculate half leaves of *N. glutinosa* (for tobacco mosaic virus) or potato (var. 11-84 of Hutton (1948) for potato Y virus) and the number of local lesions produced was compared with the number on the other half leaves inoculated with the same virus diluted with an equal quantity of water. The results are tabulated in Table 2, and demonstrate that substances in the cockroach saliva are strongly inhibitory towards tobacco mosaic and potato Y viruses.

(iii) *Inhibition by Saliva from* Nezara.—In a search for a hemipteran capable of yielding useful volumes of saliva it was found that the green vegetable bug *Nezara viridula* L. was satisfactory. The insects from the field or from laboratory colonies were mounted on microscope slides on their backs, and the stylets removed from the labium. Drops of liquid saliva accumulated at the tips of the stylets, from which it was drawn by capillarity into fine glass tubes. In this way up to 0.3 ml of *Nezara* saliva could be collected in 2-3 hr from about 100 adults. The saliva was alkaline (pH 9.5), but when mixed in equal quantities with viruses in buffered solutions the pH of the mixture was about 7·5. The technique was similar to that for *Periplaneta* saliva. The results, which are unequivocal, are given in Table 2. The saliva of *Nezara* inhibits both tobacco mosaic virus and potato Y virus.

TABLE 2

EFFECT OF INSECT SALIVA ON TMV AND POTATO Y VIRUS

Test solution, 1 part virus solution + 1 part saliva; control, 1 part virus solution + 1 part water. TMV, purified virus diluted to 10^{-4} times the concentration in tobacco leaf in M/15 phosphate buffer pH 7. Potato Y virus, 1/20 dilution of tobacco leaf infected with virus in M/15 phosphate buffer pH 7

Saliva from	Virus	Host Plant	Number of Leaves	Number of Local Lesions on	
				Test	Control
Periplaneta americana	TMV	*N. glutinosa*	8	34	277
Periplaneta americana	Potato Y	Potato (var. 11.84)	7	0	64
Nezara viridula	TMV	*N. glutinosa*	14	96	345
Nezara viridula	Potato Y	Potato (var. 11.84)	5	1	104

Differences between test and control in each experiment are highly significant.

Comparison of these results with those obtained with *Periplaneta* saliva suggests that TMV is less readily inhibited than potato Y virus; in addition, the impression was gained that *Periplaneta* saliva was more inhibitory than that of *Nezara.*

(iv) *Data on Duration of Persistence of Infectivity of Aphids.*—The above experiments demonstrate that substances capable of inhibiting certain plant viruses occur in the saliva of some insects, and suggest that viruses differ in their sensitivity to these inhibitors. This last conclusion is predicted by the modified mechanical hypothesis because of the observation that some species of aphids remain infective longer when transmitting a particular virus than do other species. Table 3 presents a summary of some of the published data on the duration of persistence of mosaic viruses in aphid vectors. Although in most instances the duration of persistence was not established within narrow limits, it will be observed that there is a wide range of variability with respect to the period during which insects remain infective, and the indications are that further work would disclose a complete series, from viruses that persist

for only a few minutes to those that persist for many days. The latter have not hitherto been included in the same group of viruses. The existence of

TABLE 3

PERSISTENCE OF NON-PERSISTENT VIRUSES IN APHID VECTORS

Times are approximate in most instances. The majority of workers have not stated at what temperatures the work was performed. Miller (1952*b*) has shown that this should be controlled in experiments dealing with persistence

Virus	Vector	Persistence		Reference
		During Feeding	During Fasting	
Cucumber mosaic	*Myzus persicae*	1-5 Min	>60 Min <120 Min	Bhargava 1951
Cucumber mosaic	*Aphis gossypii*	<20 Min	<8 Hr	Doolittle and Walker 1928
Tobacco etch	*Myzus persicae*	15 Min	>3 Hr <6 Hr	Kassanis 1941
Cabbage mosaic	*Myzus persicae*	<1 Hr	—	Kvíčala 1949
Onion yellow dwarf	*Aphis rumicis*	—	8 Hr	Tate 1940
Lettuce mosaic	*Myzus persicae*	1 Hr	>8 Hr	Kassanis 1947
Dandelion yellows	*Myzus ornatus*	1 Hr	8 Hr	Kassanis 1947
Henbane mosaic	*Myzus persicae*	Approx. 30 Min	12 Hr	Watson 1938; Watson and Roberts 1940
Potato Y	*Myzus persicae*	Approx. 20 min	12 Hr	Watson and Roberts 1940; Smith 1931
Beet mosaic	*Myzus persicae*	Approx. 3 hr	—	Severin and Drake 1948
Poison hemlock ringspot	*Rhopalosiphum conii*	>8 Hr	—	Freitag and Severin 1945*b*
Western celery mosaic	*Aphis middletoni*	7-8 Hr	—	Severin and Freitag 1938
Pea mosaic	*Macrosiphum euphorbiae*	15 Min	Max. 24 hr	Osborn 1937*a*
Citrus quick decline	*Aphis citricidus*	>24 Hr <48 Hr	—	Costa and Grant 1951; Meneghini 1948
Clover vein mosaic	*Acyrthosiphon onobrychis*	<24 Hr	—	Osborn 1937*b*
Beet yellows	*Myzus persicae*	3 Days	—	Watson 1940
Celery yellow spot	*Rhopalosiphum conii*	>12 Days	—	Freitag and Severin 1945*a*
Carrot motley dwarf	*Cavariella aegopodii*	Approx. 18 days	—	Stubbs 1948, 1952*a*

what appears to be an essentially continuous series removes one of the main reasons for separating them. It seems possible that this group of viruses comprises a series of decreasing sensitivity to the salivary inhibitors.

Evidence that the duration of persistence is influenced by feeding can be seen by comparing the persistence during continuous feeding with that during fasting, when the viruses would have less opportunity of coming in contact with a salivary inhibitor. Although the data are few (Table 3), in every instance persistence is greater in the fasting aphid than in the fed aphid. The period of survival of the virus in the feeding aphid is generally less than its survival *in vitro* (Watson and Roberts 1939). Some virus is undoubtedly wiped off the stylets during feeding, but the very short survival time of some viruses indicates that they are subjected to an additional inhibiting action. On the other hand, celery yellow spot and carrot motley dwarf viruses must be considered to be unaffected by an inhibitor.

Although most "non-persistent" viruses persist for only a short time in their vectors, Watson (1940) has shown that several plants can be infected when the vector is not permitted to feed on each for more than a few minutes. It has been demonstrated by Fenner, Day, and Woodroofe (1952) that the probability of a mosquito transmitting myxoma virus decreases with the number of feeds. A similar phenomenon has been observed with *M. persicae* transmitting potato Y virus. The experiment was performed by giving the vectors acquisition feeds of between 20 sec and 2 min and transferring them to leaves of potato (var. 11-84). The inoculation feeds were then timed with a stop-watch and the positions of feeding were marked on diagrams of the leaves. Local lesions were recorded infrequently (13 out of 321 inoculation feeds), but a significantly large proportion of the successful transmissions were produced by the first feed following the acquisition feed. Thus, nine transmissions occurred during the first feed, two during the second, one during the third, one during the fourth, and none during subsequent feeds. The rapid rate of loss of infectivity is due partly to the cleansing of the stylets during repeated feeding punctures, but it is hastened by another factor, probably the inhibiting effect of the insect's saliva. Data capable of a similar interpretation have been presented by Hamlyn (1953) who studied the transmission of cabbage black ring spot virus by *M. persicae*.

(v) *Relative Efficiency of Short and Long Acquisition Feeds.*—Further evidence of the role of the inhibitor in virus transmission should result from a comparison of the relative efficiency of short and long feeding periods on the transmission of a susceptible virus by a vector that produces an active inhibitor. This problem has been studied by several authors (Watson 1936, Kassanis 1941; Bradley 1953), although the explanation of their results has not always been apparent.

In our experiments the acquisition feeding periods of *M. persicae* were accurately timed on spinach infected with cucumber mosaic virus. "Short" feeding times were 20 sec to 2 min; "long" feeding times were 10 min to 20 min. All other factors were constant. All aphids were starved for 18 hr before the acquisition feed, and all inoculation feeds were of 15 min duration. Sixteen host plants were planted in boxes. Eight were exposed to vectors that had had short acquisition feeds and eight to vectors with long acquisition feeds, and the experiment was repeated ten times. Following "short" feeds 34 plants were

infected out of 80 exposed, whereas following "long" feeds 11 plants were infected out of 80. It is concluded that "long" acquisition feeds reduce the efficiency of transmission to one-third under the conditions of the experiment.

The results are most readily explained by the hypothesis that a product of aphid feeding reduces the efficiency of transmission of cucumber mosaic virus by *M. persicae*. It is possible that the tissues tapped during short feeds contain a higher concentration of virus than those tapped during long feeds. However, in a similar test no reduction in efficiency was found with *M. persicae* transmitting cauliflower mosaic virus, suggesting that this virus is relatively unaffected by the saliva of this aphid.

(vi) *Summary of Evidence.*—It has been proved that the saliva of certain insects is inhibitory to tobacco mosaic virus and potato Y virus. Indirect evidence that aphid saliva is inhibitory is seen in the fact that some viruses persist in their vectors for shorter periods during feeding than if the vector is fasted; and by the demonstration that short acquisition feeds produce infections with greater efficiency than long acquisition feeds in certain viruses. Those viruses in which this relationship is not found are thought to be insensitive to the salivary inhibitor.

(*c*) *What are the Effects of Fasting upon the Aphid Vectors?*

Watson (1938) found that an important difference between "persistent" and "non-persistent" viruses was their reaction to fasting of the vectors before the acquisition feeding period. Fasting had two different effects on increasing the ability of aphids to transmit non-persistent viruses; one of these was ascribed to increased "appetite," but the nature of the other was not determined. The work of Bradley (1952) provided one explanation of the effects of fasting, for he observed differences in feeding behaviour following fasting. However, the cause of the second effect of fasting has not been determined.

It was considered that the solution to this problem should help to solve the larger question of the mechanism of transmission of non-persistent viruses. A study was therefore made of the effects of fasting on the physiology of the aphid, *M. persicae,* particularly on the following: (i) digestive enzyme formation; (ii) rate of penetration of stylets; (iii) amount of plant material ingested; (iv) aphid feeding behaviour; and (v) salivary sheath formation. These will be discussed in sequence.

(i) *Digestive Enzyme Formation.*—Watson (1938) and Miller (1952*a*) suggested that viruses might be inhibited by digestive enzymes produced after a period of feeding. This hypothesis received support from the reports of Weber (1928) and Miller (1932) who, on histological grounds, believed they had observed waves of secretory activity in the aphid midgut at various times after feeding. The knowledge that trypsin inhibited some viruses (Stanley 1934; Lojkin and Vinson 1931; Kleczkowski 1944) also lent support to Watson's hypothesis.

Determinations (by the azocasein method detailed by Day and Powning 1949) of the amount of proteinase present in two series of 200 *B. brassicae,* one

group taken directly from the food plant and the other after 20 hr fasting, showed no difference between them. In neither series was the concentration of proteinase sufficient to cause measurable inhibition of virus activity, assuming that the efficiency of the enzyme from the aphid is not greatly different from that of vertebrate trypsin (Stanley 1934).

Watson (1938) reported that invertase was ineffective in inhibiting TMV. However, when it was found that easily measurable amounts of invertase were present in macerated *B. brassicae,* the effect of fasting on this enzyme was studied in an attempt to correlate possible changes in digestive enzyme concentration with the histological observations. Two per cent. sucrose was used as substrate and activity was measured by the iodine titration method as detailed by Day and Powning (1949). In duplicate controlled experiments no change was detected in invertase activity following 20 hr fasting. These results suggest that the effect of fasting was not related to changes in the production of digestive enzymes.

(ii) *Rate of Penetration of Stylets.*—Roberts (1940) showed the usefulness of studies on the depth of penetration by stylets in understanding the mechanism of virus transmission by aphids. However, it was apparent that a more rapid technique than that used by Roberts was necessary to permit comparison between normal and fasted aphids. It was known that the stylets remained extruded when aphids were removed from a leaf upon which they were feeding (see e.g. Bradley 1952), and it seemed likely that the stylets could be fixed in their natural position if a suitable fixing fluid could be found. This was accomplished by plunging the leaf upon which the insects were feeding into warm (50°C) Carnoy's fluid. The majority of aphids immediately separated from the leaf and the remainder could easily be detached. They were washed and immediately studied in 70 per cent. alcohol. The projecting parts of the stylets were then measured with a calibrated eyepiece micrometer, using a dissecting microscope with 15x oculars and a 7·5x objective, permitting measurements to 0·5 unit (7 μ).

To determine whether the results obtained by this method were a true indication of the depth to which the stylets had penetrated, measurements were made on 100 *M. persicae* taken directly from the *Datura stramonium* L. on which they were feeding, and the results compared with those of Roberts (1940) on the same aphid. The mean length of exposed stylets from our data was 150 μ, whereas the mean depth of penetration from Roberts' data varied from 136 to 143 μ, depending upon the food plant. The two methods thus give comparable results. It is possible that fixation contracts the labium, because all published illustrations, including those of Dykstra and Whitaker (1938) and Roberts (1940) in which fixation appears to be optimal, show a section of the stylets between the leaf and the labium that is not seen in the living condition. Portion of this, probably the greater part, is due to shrinkage of the plant tissues and so would not influence the measurements by the technique used in our experiments.

The effect of fasting on the rate of penetration was then studied by measuring the lengths of exposed stylets of aphids fasted for 10 min, 60 min, and

20 hr before various feeding periods. The results (Table 4) show (1) that *M. persicae* can penetrate as far after 30 min feeding at laboratory temperatures as they do when left to feed at will on the plants, and (2) that the differences observed in the depth of penetration following fasting were too small to account for the recorded differences in efficiency of virus transmission.

TABLE 4

MEAN DEPTH (μ) OF PENETRATION OF STYLETS OF *M. PERSICAE* INTO LEAF OF CHINESE CABBAGE AS AFFECTED BY DURATION OF FASTING

Number of stylets measured was 660. Depth of penetration of 100 *M. persicae* feeding on Chinese cabbage was 111 μ

Fasting Period	Feeding Periods (min)		
	5	10	30
10 Min	52±3*	77±3	100±3
60 Min	65±4	82±4	112±4
20 Hr	62±2	62±3	101±3

*This figure includes some insects whose stylets were not retracted following removal from the host plant (see Section (iv) below).

Efficient transmission of cauliflower and cucumber mosaic viruses was obtained after 2-min acquisition feeds, and so it was thought desirable to perform a carefully controlled experiment to determine the depth to which normal and fasted *M. persicae* can penetrate during accurately timed 2-min feeding periods on spinach. *M. persicae* starved 18-21 hr penetrated 33 μ into the leaf (minimum 0·0 μ, maximum 74 μ) whereas insects removed directly from the host plant penetrated 36 μ (minimum 0·0, maximum 80 μ) in the same period. The results demonstrate that there is no significant difference between the treatments and that there is a rapid rate of penetration during the early stages of probing. Quite frequently the aphids penetrated in 2 min through the epidermal cells into the underlying tissues.

(iii) *Effect of Fasting on the Amount of Material Ingested.*—Results of an examination of the possible effects of fasting on the amount of plant material ingested have been reported in a study of the feeding of *M. persicae* (Day and Irzykiewicz 1953). In this work radiophosphorus was incorporated in plant tissues and in artificial diets presented through plastic membranes. Although feeding periods were of necessity longer than desirable to detect possible effects of fasting, the results gave no indication that fasted insects ingested an amount different from that ingested by non-fasted aphids.

(iv) *Effect of Fasting on Aphid Feeding Behaviour.*—Bradley (1952) has shown that aphids transferred directly from one plant to another are only very rarely able to feed immediately. This is because the stylets protrude from the labium when the insect is removed from a plant on which it has been feeding

and it takes time for these stylets to be ensheathed again. Until this has occurred the stylets cannot be reinserted into a plant. The normal behaviour is for the aphid then to probe the leaf lamina. It then moves off and repeats the operation until it comes to rest, generally on a vein where it feeds for a longer time. After periods of starvation the probing is frequently omitted and feeding for an extended period may occur on the leaf lamina.

It will be apparent that the time required for aphids to ensheath their stylets is important in experimental technique, because most workers have removed aphids forcibly from one plant before transferring them to a second. The number of insects with protruding stylets at various times after removal from a plant is shown in Table 5 for *M. persicae*, *B. brassicae*, and *M. euphorbiae*.

TABLE 5

DATA ILLUSTRATING THE RATE OF LABIAL EXPANSION TO COVER STYLETS AFTER REMOVAL FROM THE HOST PLANT AT 22°C

For each time 200 aphids counted, except for *M. euphorbiae*; 100 were counted for zero time and 50 after 2 min

Time (min)	Percentage With Stylets Protruding		
	M. persicae	*B. brassicae*	*M. euphorbiae*
Immediate	74·5	87·0	70·0
1	43·5	—	—
2	33·5	—	0·0
5	30·0	16·5	—
10	21·5	—	—
15	8·0	7·5	—
30	1·5	—	—

The observation that 8 per cent. of *M. persicae* and *B. brassicae* were still unable to feed 15 min after their removal from the host plant explains part of the marked increase in transmission rate reported by many authors following short periods of fasting. This explanation does not hold for *M. euphorbiae*, which is able to ensheath its stylets very readily.

One result of 30 min fasting before an acquisition feed is therefore that the insects are able to feed immediately and in many instances do so as soon as they come to rest upon a leaf.

(v) *Effects of Fasting on Salivary Sheath Formation.*—The effect of fasting described in the previous section, namely the less frequent probing by fasted aphids, can be overcome by watching the feeding behaviour of vectors with a lens. If this is done, it is found that fasting for from 30 min to 1 hr still has an effect on the ability of *M. persicae* to transmit cucumber mosaic virus. Following an acquisition feed of 1-2 min and inoculation feeds of 15 min, 40 out of 94 attempted transmissions were positive for the fasted aphids. This may

be compared with 27 infections out of 96 for attempted transmissions by aphids removed directly from the food plant but which were checked for their ability to feed within 5 min of being placed on the infective plant. This increase due to fasting is low compared with that reported by previous workers because of the additional controls on feeding, but the difference between the two groups is significant. Increasing the period of fasting to 18 hr does not significantly alter the ratio of infections, viz. 24 out of 96 for fasted and 12 out of 96 for non-fasted aphids. These results cannot be used for comparison with the previous series because of differences in the source plant.

The remarkable effect of short periods of fasting (30-60 min) is readily explained by the suggestion that the inhibitor disappears from the mouth-parts or is diminished during these short periods of fasting.

These results agree with the observations of Bradley (1952), Sylvester (1950*a*), Bhargava (1951), Miller (1952*a*), and others on the effects of fasting on virus transmission. All these workers have shown a marked effect of fasting when non-fasted insects were compared with those fasted from 30 min to 1-3 hr. Fasting for periods in excess of 1-3 hr produced no further improvements in transmission. These results are in distinction to those of Watson (1938; 1946) and Watson and Roberts (1939) who reported, in all of a number of experiments, further improvement in transmission efficiency when aphids were made to fast for periods up to 24 hr. In every test the increased efficiency was slight but the probability of its being due to chance becomes very small when all experiments are considered together. A further consequence of fasting must therefore be operative. An explanation of this second effect was therefore sought.

On the hypothesis that saliva contained the virus inhibitor, it seemed possible that increased vector efficiency following long periods of fasting might be caused by resulting changes in salivary production.

Efforts were therefore made to determine the rate of production of the salivary sheath in normal and fasted insects. No sheath could be demonstrated in plant tissues by the use of Millon's reagent unless aphids (*M. persicae* on Chinese cabbage and *B. brassicae* on wild mustard) had fed for more than an hour. It was found, however, that a sheath produced in a liquid medium could be detected more rapidly. When the liquid contained very dilute methylene blue the dye was taken up by the sheath and could readily be seen. This technique permitted the observation of salivary sheaths within a minute after the plastic membrane had been punctured. This was accomplished both by non-fasted aphids and aphids fasted up to 24 hr. Starvation for 48 hr, however, resulted in feeding occurring before salivary sheaths were formed. In fact, a sheath was never seen to be formed during the first puncture after these extended periods of starvation.

This visible result of starvation suggested that shorter periods might have some effect on salivary components that was not observable by the crude method of observing salivary sheath formation. Weber (1928) has already reported marked changes in the histology of salivary glands of *Aphis fabae* L. resulting from a short feed following a period of fasting. Attempts to confirm this obser-

vation with *M. persicae* and with *M. euphorbiae* have been unsuccessful. A comparison was made of the histology of salivary glands of aphids treated in the following manner: (*a*) taken from the plant, (*b*) fasted 1 hr, (*c*) fasted 2 hr, (*d*) fasted 3 hr, (*e*) fasted 18 hr, (*f*) fasted 18 hr, fed 15 min, (*g*) fasted 18 hr, fed 1 hr, (*h*) fasted 18 hr, fed 2 hr, (*i*) fasted 18 hr, fed 3 hr, (*j*) fasted 18 hr, fed 6 hr. Careful comparison of photographs of 10-μ sections stained in Mallory's triple stain failed to reveal any differences attributable to the treatments. The periods of fasting and feeding studied should have permitted the confirmation of Weber's results, but no changes of the kind he described were noted in sections of approximately 60 insects examined. No instance was seen of cellular depletion as marked as that illustrated in Weber's Plate 12, Figure 35 (*b*). Some changes, apparently connected with a secretory cycle, were noted in the cells, but they were unrelated to the experimental treatments. It seems unlikely that *A. fabae* differs so much from *M. persicae* and *M. euphorbiae.* We conclude from our observations that the histology of the salivary glands provides no evidence of changes in the components of the salivary secretions due to fasting or feeding.

(vi) *Summary.*—The conclusions from the work reported in this section may be summarized thus: Fasting was found to have no marked effect on digestive enzyme formation, rate of penetration of stylets, or amount of material ingested. On the other hand, the demonstrable effect of fasting on feeding behaviour, stylet ensheathment, and the formation of saliva would seem to be sufficient to explain the effect on virus transmission. The hypothesis that the greatest effect of fasting is on the salivary inhibitor is supported by these results.

(*d*) *What Explanations are Available for the Observed Specificity in Virus-Vector Relationships?*

(i) *The Occurrence of Specificity.*—A review of published data makes it at once apparent that vector specificity, in the sense in which that term is applied in the leafhopper-borne viruses, does not exist in the non-persistent aphid-borne viruses. The number of examples of aphids being incapable of functioning as vectors is very small in comparison with the number of positive transmissions. *Doralis rumicis* (L.) is, however, exceptional. It has been shown to be incapable of transmitting five different non-persistent viruses. It transmitted beet mosaic once in some hundreds of experiments (Severin and Drake 1948) and transmitted western celery mosaic in only 2·2 per cent. of tests (Severin and Freitag 1938); these performances indicate an unusual inability to act as a vector and suggest that this species would repay careful study. A comparable, though less marked result was reported by Watson and Roberts (1939) for *M. euphorbiae* (= *M. gei*). They stated that "*M. gei* is a poor vector because its capacity for inactivating the viruses is greater," and their final conclusion was that "the relative efficiency of the vectors varied with the different viruses, indicating that their degree of success depended upon several interacting factors . . . " one of which is "the capacity of the vector for inactivating the virus."

Although vector specificity is not a feature of the transmission of non-persistent viruses, there are marked differences in the efficiency of various vectors. Such differences have been reported many times and some examples are summarized in Table 6.

TABLE 6

RELATIVE EFFICIENCY OF VARIOUS SPECIES OF APHIDS IN THE TRANSMISSION OF SOME NON-PERSISTENT VIRUSES

Virus	Aphis Species in Order of Vector Efficiency	Reference
Cucumber mosaic	*M. persicae* > *M. circumflexus* > *M. euphorbiae*	Watson and Roberts 1939
Henbane mosaic	*M. persicae* > *M. circumflexus* > *M. euphorbiae*	Watson and Roberts 1939
Cauliflower mosaic	*M. pisi* > *A. apii* > *M. circumflexus* > *C. capreae* > *M. solani* > *A. apigraveolens* and others	Severin and Tompkins 1948
Beet mosaic	*A. apigraveolens* > *A. apii* > *A. gossypii* > *C. capreae* > *A. pomi* > *B. brassicae*	Severin and Drake 1948
Beet mosaic	*M. persicae* > *M. solani* > *A. apii* > *M. circumflexus*	Sylvester 1952
Pea mosaic	*M. persicae* > *M. euphorbiae* = *D. rumicis*	Chamberlain 1936
Lettuce mosaic	*M. pelargonii* > *M. persicae*	Dias 1951

It should be stressed that in many instances the number of successful transmissions was insufficient to permit too rigid comparisons, but it is clear that *M. persicae* is frequently one of the most efficient vectors in addition to being one of the most common vectors. It is not, however, invariably the most efficient vector. These differences between vectors require explanation.

(ii) *Effect of Host Plant on Salivary Sheath Formation.*—Stylet tracks made by *M. persicae* in a series of different host plants were observed after staining in Millon's reagent (for the method, see Day, Irzykiewicz, and McKinnon (1952)). In *Datura,* carrot, and tomato (not a preferred host) the sheaths were fine and hairlike, but in potato the sheaths were much more conspicuous and there was a conspicuous darkening of cell walls in the vicinity of the puncture, with a noticeable increase in tissue destruction. This observation lends support to the view that the aphids react differently with respect to saliva production to different host plants.

The related problem of visibly different feeding tracks being produced by several aphid species attacking a single host plant was studied by comparing the salivary sheaths of *M. persicae* and *B. brassicae* on Chinese cabbage. Well-marked differences in the tracks were readily discernible although the differences were not as striking as those resulting from feeding tracks formed by a series of leafhoppers on *Malva* (Day, Irzykiewicz, and McKinnon 1952).

(iii) *Specific Differences in Aphid Behaviour.*—Comparisons of feeding behaviour of *M. persicae* and *B. brassicae* on swede turnips showed that the duration of each prefeeding probe was much greater for the latter than it was for *M. persicae.* A comparison was then made between the feeding behaviour

of *M. persicae* and that of *M. euphorbiae*. Non-fasted *M. euphorbiae* do not feed readily when transferred to a new host, and may remain in a feeding position for 30 min without puncturing the leaf. They generally probe more often before settling to feed than does *M. persicae*; also they probe more deeply but withdraw their stylets very much more easily (see Table 5). These readily observable differences in behaviour suggest that the efficiency of virus transmission may differ from one species to another for this reason alone. It seems probable that the "vector-host plant compatibility factor" of Simons and Sylvester (1953) is explicable by these differences in behaviour of aphids on various food plants.

A comparison was made of the ability of *M. persicae* and *M. euphorbiae* to act as vectors of cucumber mosaic virus. Watson and Roberts (1939) found that *M. persicae* transmitted this virus 20 times in 105 attempts, whereas *M. euphorbiae* transmitted twice in 105 attempts. In our experiment an attempt was made to eliminate the effect of the differences in behaviour mentioned in the previous paragraph. Aphids were starved overnight. The acquisition feed was carefully timed and watched with a 10x magnifier. Only insects that fed for 2 min were used. Those that did not obtain their acquisition feed within 5 min of being placed on the infected plant were discarded. Transmission was to spinach seedlings in the four-leaf stage. Half the seedlings in flats containing 16 plants were exposed to *M. persicae* and half to *M. euphorbiae*, and the test was repeated eight times. *M. persicae* transmitted 21 times in 64 attempts, whereas *M. euphorbiae* transmitted five times in 64 attempts. Thus *M. euphorbiae* was four times less efficient as a vector of cucumber mosaic virus than *M. persicae*, in spite of attempts to provide optimum conditions for transmission.

The modified mechanical hypothesis suggests that these differences in efficiency of transmission may be largely accounted for on the basis of differences in the salivary inhibitors, but other factors mentioned in the previous paragraphs may also be operative.

(iv) *Summary.*—It will be apparent from the above discussion that the occurrence of specificity and differences in vector ability are not arguments against the hypothesis that non-persistent viruses are transmitted mechanically. There are, in fact, many possible theoretical explanations for most of the examples of specificity observed, and there is evidence for some of these possibilities.

III. Discussion

The results of the experiments described in the preceding section tend to substantiate the modified mechanical hypothesis of the mechanism of transmission of aphid-borne non-persistent viruses advanced in the Introduction. Where the experimental evidence is inadequate there is very considerable difficulty in performing the crucial experiments, owing to the small size of the aphid stylets and attendant difficulties. No results have been obtained that conflict with the hypothesis.

In 1940 Watson and Roberts contended that the hypothesis of mechanical transmission rested on only two arguments, both of which they believed were

capable of an alternative explanation. Since then additional arguments have become apparent, and the modified mechanical hypothesis is now based on the following observations: (1) The short transmission cycle, (2) the absence of a latent period, (3) the short duration of retention of the virus and the loss of infectivity in successive inoculation feeds, (4) the absence of vector specificity, (5) the ease with which these viruses can be transmitted mechanically, and (6) the absence of retention of the virus following a moult.

These six points taken together seem to permit of no explanation other than that the viruses are transmitted mechanically.

(*a*) *Difficulties in Application of the Hypothesis*

However, certain difficulties still remain; Bawden and Kassanis (1947) found that some individuals of a colony of *Myzus ornatus* Laing could transmit potato Y virus, whereas most members of the colony could not. It is possible that the few vectors produced less salivary inhibitors than the majority of the colony, but apart from the role of chance, no other explanation capable of experimental examination can be offered for this report. The report is, however, not as unusual as may at first appear. The low efficiency of some vector species found by many workers is probably attributable to the same phenomenon.

Hoggan (1931) found that certain species of aphids transmitted tobacco mosaic virus from tomato to tomato, but the same species were unable to transmit from tobacco to tobacco, even though this plant had a higher virus content than the tomato. The result has never been confirmed, but, if substantiated, may possibly be explained on the basis that the aphids produce more salivary inhibitor in response to one species of food plant than they do to another. Alternatively the virus may be less aggregated and hence more accessible in the tomato, for Black, Morgan, and Wyckoff (1950) have demonstrated that TMV may be greatly aggregated in infected tobacco.

Another report not easily explained is that of Sylvester and Simons (1951) who found that the green peach aphid is a better vector of *Brassica nigra* virus when fed on mustard (*Brassica juncea*) but that the false cabbage aphid is a more efficient vector when fed on pak choi. This interesting observation could be accounted for if each species of aphid produced different amounts of saliva or salivary components on the two hosts.

The inefficiency of the transmission of TMV and potato virus X by aphids requires explanation, because these viruses are easily transmitted mechanically and have in fact been transmitted by biting insects (Walters 1952). They may be more susceptible to salivary inhibitors than most non-persistent viruses, but this seems unlikely in view of the results reported above on *Nezara* saliva inhibition of TMV. Although both rod and spherical-shaped viruses are aphid-transmissible, TMV and potato X virus are somewhat longer than most. It may thus be difficult for them to attach to the aphid stylets. Furthermore, both have a marked tendency towards end-to-end aggregation. This aggregation occurs *in vitro,* and also in the host cell, and may add to the difficulty of acquisition by aphids. There are thus several possible reasons for the failure of TMV

and potato X virus to be transmitted by aphids and this failure is doubtful evidence for discounting the modified mechanical hypothesis.

(*b*) *Re-evaluation of Classification of Aphid-borne Viruses*

Watson (1946) suggested that a basis for separating aphid-borne viruses into two groups might be their response to preliminary fasting, but pointed out the difficulties inherent in the use of this and similar criteria. On the basis of the suggestions in the present paper it is desirable to find alternative terms for what now appear to be the logical groupings of aphid-borne viruses and the terms "vector-direct" and "vector-latent" are suggested.

When a virus is transmitted on the mouth-parts, it is generally readily sap-transmissible, fasting the vector usually increases its efficiency, there is never a long latent period in the vector, and the virus does not multiply in the vector. Many such viruses reach relatively high concentrations in the mesophyllic tissues and cause mosaic diseases in plants. Those belonging to this group are vector-direct viruses. When viruses are transmitted by the mechanism involving ingestion, passage through the gut, into the haemocoele, and reinjection with the saliva, there is often a long latent period between the acquisition feed and a successful inoculation feed; many of these viruses are specific to a relatively few species of vectors and they are generally not sap-transmissible, fasting the vector usually has no effect on its efficiency; some of these viruses are confined to the vascular tissues of the host plants, they may multiply in their vectors and they may be designated vector-latent viruses. This view of aphid transmission brings these virus-vector relationships into line with mosquito-transmitted viruses. In these, rabbit myxoma is a typical vector-direct virus, whereas yellow fever is the classical example of a vector-latent virus.

There is no fundamental objection to the suggestion that a vector that normally transmits by the biological mechanism may occasionally contaminate its mouth-parts with infective virus, and so cause transmission. In some instances in which the virus is resistant and infective when introduced into the epidermal or subepidermal tissues it is possible that the vector habitually transmits by both mechanisms; but, so far, no example of this twofold mechanism of transmission is known.

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