dextran (Pharmacia Fine Chemicals; molecular weight, 2 million).

I studied the breakdown of a 2 percent solution of this substrate viscometrically (*4*). The viscosity measurements were very accurate, and the flow time varied by only 1 or 2 seconds (about 0.2 percent).

In order to determine the enzyme activity in the tissues, 20 or 30 sections (1 cm long) taken 4 mm below the tip of dark-grown coleoptiles (2 to 3 cm long) of *Avena sativa* were ground. The pellets from the twice centrifuged (2300*g*) and washed material were incubated at 45°C for four or more hours at *p*H 5.0 with the specific substrate. This method has been described (*1*).

The decrease in viscosity is about 15 percent for normal coleoptiles and is more after longer incubation (Table 1). With a suspension of 0.5 μg of purified commercial dextranase per milliliter, a 20 percent decrease in viscosity occurs after 3 minutes and a decrease of more than 80 percent after 30 minutes or at higher enzyme concentrations. If the substrate is incubated with boiled pellets, no decrease of viscosity occurs. If the substrate is incubated with pellets of sections from coleoptiles that have been decapitated for about 2.5 hours, in order to lower the auxin concentration in the tissues, a much lower decrease in viscosity (5 percent), or none at all, occurs (Table 1).

In order to exclude the possibility that decapitation might have caused the observed changes in enzyme activity, an experiment was carried out in which sections 1 cm long were taken 4 mm below the tips of coleoptiles which had all been decapitated—to lower their auxin content—for 1.5 to 2.5 hours previously. Twenty sections from these plants were placed for 1.5 hours in a solution of auxin (10 parts per million), and 20 control sections were placed in water. After the hormone treatment, pellets were prepared from the sections.

In all cases, the hormone-treated sections had a higher enzyme activity than the untreated sections (Table 2), although the differences between the two groups were smaller than those between normal and decapitated coleoptiles.

This seems to justify the conclusion that the enzyme activity is sensitive to the presence of the hormone. This is the first enzyme activity found in coleoptiles of *Avena* which increases with higher auxin content, contrary to what has been found with other enzymes (*1*). Furthermore, this enzyme activity is affected by the hormone within a short period commensurate with the short reaction time of auxin-induced elongation.

Table 2. Dextranase activity in pellets prepared from 20 sections of coleoptiles of *Avena* as judged by viscosity of dextran solutions after 4 hours of digestion. Half of the pellets were prepared from sections of coleoptiles which were treated 1.25 hours in auxin solutions (10 ppm). Activity expressed as flow in seconds. Numbers in parentheses represent percent decrease in flow time as compared with control substrate. In A, B, and D the coleoptiles were decapitated 1.5 hours before the sections were cut. In C the coleoptiles were decapitated 2.5 hours before the sections were cut. In D the sections were treated in the dry state with auxin (10 ppm) in talcum powder.

Type of pellet	Flow time of 2% dextran after coleoptile treatment			
	A (sec)	B (sec)	C (sec)	D (sec)
None	410	410	475	352
From hormone-treated sections	367 (10.5)	383 (7.0)	457 (4.0)	317 (10.0)
From untreated control sections	391 (4.7)	392 (4.7)	470 (1.0)	332 (5.3)

If a pure natural dextran can be broken down by the activity of this enzyme in coleoptiles, the same might also happen to dextran-like substances which might be found in the cell walls of these coleoptiles. To determine whether or not any substances occur in these cell walls which can be broken down by dextranase, cell wall material (carefully washed pellets) was subjected to the action of pure dextranase. A chromatographic analysis of the hydrolyzates showed that a breakdown of some cell wall components had indeed occurred. The chromatogram was very similar to that of the hydrolyzate of the natural dextran obtained with pure dextranase. The main spots in both cases corresponded to isomaltose and isomaltotriose (with mobilities of 0.52 and 0.26 of that of pure glucose), the sugars typically produced in the breakdown of dextrans by dextranase (*5*). Thus, dextran-like substances (eventually combined with other compounds) on which dextranase can act must be present in the cell wall. The implications of these findings for the problem of the mechanism of cell elongation are obvious (*6*).

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Barley Yellow Dwarf Virus: Phenotypic Mixing and Vector Specificity

Abstract. *Although the aphid* Rhopalosiphum padi *does not regularly transmit the MAV isolate of barley yellow drawf virus from singly infected oats, it often transmits MAV, together with the serologically unrelated RPV isolate, from plants doubly infected by MAV and RPV. Vector specificity of the virus isolates appears to be a function of the virus capsid. Some MAV nucleic acid becomes coated with RPV capsid protein during simultaneous synthesis of the two isolates in the doubly infected plant.*

Incorporation of nucleic acid of one virus into particles containing the protein capsid of another virus, called phenotypic mixing, has been mentioned (*1*) as a possible explanation for cases in which aphid transmission of one plant virus depends on the presence of a second in the source plant (*2*). Evidence for this possibility has been lacking despite many examples of phenotypic mixing for bacterial and animal viruses (*3*). This report presents evidence for the role of virus capsid protein in vector specificity and for the phenotypic mixing between two serologically unrelated isolates of

Table 1. Virus transmission by aphids fed through membranes on mixtures of virus preparations (made from oats doubly infected by the RPV and MAV isolates of barley yellow dwarf virus) and 1 of 3 antiserums, and identification of the isolates recovered from the plants that became infected by means of the membrane-fed aphids.

Antiserum to:	Plants infected/plants infested with:		Plants infected* with:		
	R. padi	*M. avenae*	RPV	MAV	Both
MAV	43/50		23	0	13
MAV		0/51			
RPV	0/51				
RPV		50/50	0	16	0
HO	34/51		11	0	11
HO		50/50	0	15	0
None	37/42		6	1	10
None		38/38	0	12	0
Aphid controls	0/51	0/51	0	0	0

* Identification of 40 cases of infection with RPV was based on virus transmission to all of 141 plants by *R. padi* and to none of 141 plants by *M. avenae*; identification of 44 cases of infection with MAV was based on virus transmission to 3 of 168 plants by *R. padi* and to all of 167 plants by *M. avenae*; identification of 34 doubly infected plants was based on virus transmission to all of 144 plants by *R. padi* and to all of 144 plants by *M. avenae*. Recovery of MAV from the doubly infected plants was further confirmed by tests in which virus was transmitted to 3 of 66 plants by *R. padi* and to all of 65 plants by *M. avenae*. The antiserums were specific for the MAV isolate, the RPV isolate, or for a concentrated preparation of healthy oats (HO) used as a control.

barley yellow dwarf virus (BYDV), a circulative plant virus that is usually transmitted to plants only by aphids (*4*).

From singly infected Coast Black oats (*Avena byzantina* K. Koch), the aphid *Rhopalosiphum padi* (Linnaeus) regularly transmits the RPV isolate of BYDV but not the MAV isolate, which in turn is transmitted specifically by another aphid, *Macrosiphum avenae* (Fabricius). Although this specificity of the two virus isolates is relative, it has remained a consistent property (*5*). From leaves of oats doubly infected by both RPV and MAV, or from extracts of doubly infected leaves, *M. avenae* transmits only MAV; but *R. padi* often transmits virus subsequently transmissible by both aphid species (*6*). Tests with antiserums specific for each isolate have now shown that this apparent loss of specificity for virus transmitted by *R. padi* results from transmission of both RPV and MAV from doubly infected leaves. Thus, *R. padi* can transmit MAV from doubly infected leaves but not from singly infected ones, a phenomenon similar to other cases in which aphid transmission of one plant virus depends on the presence of a second (*2*).

When aphids feed through Parafilm on concentrated preparations of RPV, MAV, or mixtures of the two, each aphid species can acquire (and transmit to oats) only the isolate that it transmits specifically from singly infected leaves (*5*, *6*). If the virus preparations are mixed with antiserums, however, transmission of either isolate is blocked by the homologous, but not by the heterologous, antiserum (*7*). Since the transmission pattern was the same when aphids fed on virus preparations made from plants doubly infected by RPV and MAV as when they fed on leaves (*6*), I used such virus preparations in tests with antiserums. Transmission by *M. avenae* of MAV from preparations made from doubly infected plants was blocked when the virus had been mixed with MAV antiserum; MAV was considered to be neutralized. From the same preparation, however, *R. padi* transmitted virus to plants that subsequently often contained both RPV and MAV. Thus, treatment with MAV antiserum of virus preparations from doubly infected plants prevented transmission of MAV by *M. avenae* but not by *R. padi*. Apparently, during simultaneous synthesis of MAV and RPV in the doubly infected plant, some MAV nucleic acid becomes coated with RPV capsid protein and functions in the interaction of virus and vector as RPV but functions in the plant as MAV.

In one kind of experiment, a partially purified preparation (1.0 to 10.0 μg of virus in 1.0 ml) from doubly infected plants was mixed with an equal volume of antiserum (diluted 1 : 10), specific for either MAV, RPV, or a concentrate of healthy oats as a control. Each mixture was kept at 37°C for 30 minutes and stored overnight at 4°C. The mixture was centrifuged at low speed. The supernatant was diluted with an equal volume of 40 percent sucrose in neutral 0.1*M* potassium phosphate buffer, divided into two portions, and fed, in parallel tests, to *R. padi* and *M. avenae*. After the aphids had fed through stretched Parafilm on the treated virus preparations for about 18 hours at 15°C, they were moved to seedlings of Coast Black oats (10 aphids per seedling) for 5 days at 21°C. Plants that became infected during the following 4 weeks were then used in subsequent comparative transmission tests with the two aphid species to determine which of the virus isolates had been transmitted from the treated virus preparations.

Results of tests on each of five dilutions of virus in two experiments were all in agreement (Table 1). *Macrosiphum avenae* transmitted virus from every preparation except those treated with MAV antiserum. All tests on 43 of the plants that became infected showed that *M. avenae* recovered only MAV from the virus preparations. *Rhopalosiphum padi* transmitted virus from each of the treated preparations except those incubated with RPV antiserum. In each test with *R. padi* some of the infected plants developed severe symptoms characteristic of those doubly infected by both RPV and MAV; subsequent comparative tests confirmed that *R. padi* had transmitted both RPV and MAV to some plants. These included 13 of 36 plants that became infected after *R. padi* had fed on the virus preparations incubated with MAV antiserum. Despite neutralization of MAV, *R. padi* transmitted virus that produced MAV infections in about one-third of the plants tested.

In another kind of experiment, aphids were injected with virus preparations instead of being allowed to acquire virus by feeding (*8*). Virus (10 μg/ml) from doubly infected plants that had been incubated with MAV antiserum was used in each of two experiments. None of 24 plants infested with *M. avenae* became infected, but 20 of 24 plants infested with *R. padi* in parallel tests became infected. Tests on 17 of the infected plants showed that 10 were infected only by RPV, because *R. padi* transmitted virus from them to 30 of 30 plants whereas *M. avenae* transmitted virus to 0 of 30. The other 7 plants, which developed severe symptoms, were infected by both RPV and MAV since both aphid species transmitted virus from these plants to 21 of 21 assay plants. None of 34 plants infested as controls became infected. Thus, *R. padi* had transmitted MAV to about one-third of the plants despite neutralization of the virus preparations with MAV antiserum.

Density-gradient centrifugation was used in combination with tests of in-

fectivity in a third kind of experiment to estimate relative absorption of virus by each antiserum. Each of three samples of virus from doubly infected plants (12 μg in 0.4 ml) was mixed with 0.6 ml of a 1 : 10 dilution of antiserum against MAV, against RPV, or against healthy oats. The mixtures were incubated for 40 minutes at 37°C, stored at 4°C for about 19 hours, centrifuged at low speed to remove the precipitate, layered on a sucrose gradient column, centrifuged for 3.5 hours, and scanned with the ISCO density gradient fractionator with the sensitive (0.5 optical density) scale and an external recorder (*4*). The peak region of each column was collected (total 3.0 ml), divided into two portions, and used in parallel assays with both aphid species feeding through membranes.

The low concentration of virus precluded precise estimates, but less virus remained after absorption with the RPV antiserum than remained after absorption with MAV antiserum; total unabsorbed virus in the two treatments was about equal to that of the control (Fig. 1). In the infectivity assays, *M. avenae* transmitted virus to all test plants from all samples except from the one absorbed with MAV antiserum. Subsequent tests on the 24 plants infected by *M. avenae* showed that only MAV had been recovered (Fig. 1). *Rhopalosiphum padi* transmitted virus from two samples but not from the sample treated with RPV antiserum; some plants in each group contained both RPV and MAV, regardless of whether the sample had been incubated with control antiserum for healthy oats or that for MAV (Fig. 1). Again the MAV antiserum prevented transmission of MAV by *M. avenae* but not by *R. padi.*

Identification of MAV recovered from the treated virus preparations in these experiments was based on relative severity of symptoms, on subsequent specific transmission by *M. avenae*, and on serological tests. Because of low virus titer, serological tests could not be carried out on individual plants. Some of the infected test plants were grouped together and used to make a concentrated preparation. In one experiment, for example, the plants used were those infected by means of *M. avenae* in subsequent comparative transmission tests which were made of plants to which *R. padi* had transmitted virus from the original treated virus preparation. One concentrated preparation was made from 171 g of tissue that included 51 plants from 17 tests of plants infected by means of *R. padi* that had fed on virus (from doubly infected plants) treated with MAV antiserum. A parallel preparation was made from 99 g of tissue from 30 plants of tests in which the original preparation had been mixed with the control antiserum for healthy oats. In Ouchterlony agar double-diffusion tests the preparations reacted faintly with MAV antiserum. A few drops of each preparation were diluted for use in membrane feeding tests. *Macrosiphum avenae* transmitted virus from both preparations to 12 of 12 plants when the preparations were mixed with RPV antiserum, but to none of 12 plants when the preparations were mixed with MAV antiserum. None of 12 control plants became infected. Thus, MAV recovered by *R. padi* from treated preparations made from doubly infected plants produced infections of MAV indistinguishable from those caused by MAV from single infections.

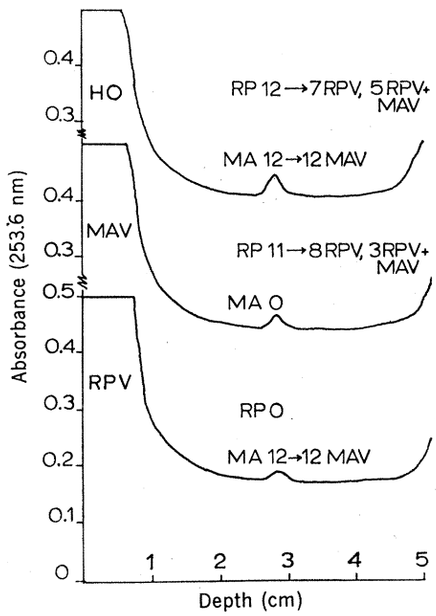

Fig. 1. Scanning patterns and infectivity tests on the same sample from each of three tubes. About 12 μg of virus from doubly infected plants was absorbed with an antiserum for healthy oats (HO), an antiserum for MAV, or an antiserum for RPV, and centrifuged at 23,000 rev/min for 3.5 hours on a sucrose gradient (SW 25.1 rotor, Spinco model L). Numerals above the peaks are numbers of plants that became infected out of 12 infested with about 10 aphids of *Rhopalosiphum padi* (RP) or *Macrosiphum avenae* (MA) that had fed through membranes on the portion of the gradient that contained the unabsorbed virus. Data after the arrows are numbers of infected plants subsequently found to contain the virus isolate or isolates shown. The 24 plants infected only by MAV were identified in comparative aphid transmission tests in which *R. padi* transmitted virus to 19 of 105 plants and *M. avenae* transmitted virus to 105 of 105. Fifteen plants were infected only by RPV because *R. padi* transmitted virus from them to 45 of 45 plants but *M. avenae* transmitted to 0 of 45. The eight doubly infected plants were identified by severe symptoms and virus transmission by both aphid species to 24 of 24 plants. None of 42 plants infested as controls in these experiments became infected.

The possibility that treatment with MAV antiserum somehow alters MAV in vitro to make it transmissible by *R. padi* was also investigated. Different portions of the same preparation of MAV (10 μg) were mixed separately with each of the three antiserums, diluted with sucrose, and used in membrane feeding tests. *Macrosiphum avenae* transmitted virus to 12 of 12 plants after feeding on the preparations incubated either with serum prepared against the RPV isolate or serum prepared against healthy oats, but to none of 12 plants after feeding on the sample treated with antiserum against MAV. *Rhopalosiphum padi* did not transmit virus to any of 36 plants in parallel tests. None of 24 plants infested as controls became infected. This experiment agrees with those of previous tests that showed failure in attempts to alter MAV in vitro to make it transmissible by *R. padi* (*5*).

These results suggest a basis for some of the variation encountered among naturally occurring isolates of BYDV, they suggest how "new" virus variants or vectors could function in nature where mixed infections are not uncommon, and they point to the interaction between virus capsid protein and specific sites or tissues within the aphid as a major basis for BYDV-vector specificity. The results also suggest that phenotypic mixing could explain other instances in which transmission of a plant virus by aphids occurs only when the virus is accompanied by a second virus (*1, 2*). The results suggest that the extent of phenotypic mixing for BYDV might differ somewhat from that in animal and bacterial viruses. Although quantitative data are only preliminary, the anomalous BYDV particles appear to be nearly or completely coated by protein of one virus isolate (RPV) in contrast to many other examples where phenotypically mixed virions are doubly neutralizable because they contain pro-

tein from more than one virus (*3*). Another distinctive feature of this system is that mixing has been detected only in one direction.

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Metabolic Dependence of Fast Axoplasmic Transport in Nerve

Abstract. *Fast axoplasmic transport, shown in cat sciatic nerves by a crest of labeled activity after injection of the L7 ganglion with [³H]-leucine or [³H]-lysine, was stopped within 15 minutes after death of the animals by bleeding. If the sciatic nerves were removed from the animals and placed in a chamber supplied with oxygen at 38 degrees centigrade, fast transport was sustained. Transport was rapidly blocked in similar in vitro preparations when the nerves were kept in a nitrogen environment. Fast axoplasmic transport is closely dependent upon oxidative metabolism.*

A fast transport system moving materials outward in nerve fibers at the rate of 410 mm/day was shown by the distal displacement of a crest of labeled activity in cat sciatic nerves after injection of the lumbar seventh (L7) ganglia with the precursor [³H]-leucine (*1*). In a more extensive series of experiments the rate was 401 ± 35 mm/day (*2*). The cell bodies are likely to be the major if not the sole, site of incorporation of precursor into labeled soluble proteins, polypeptides, and small particulates carried down inside the nerve fibers (*2, 3*).

Transport is not brought about by an active force exerted from the cell bodies or by diffusion from the somas. This was shown by eliminating the contribution of the cell bodies either by a ligature placed just distal to the ganglia or by its destruction after the ganglia were injected with [³H]-leucine. In such experiments a peak of labeled materials moved down the nerve fibers at the same rate as that present in control nerves (*2*).

If, as indicated by such results, fast axoplasmic transport is an active process present locally in the fibers, we would expect it to depend on metabolism. A similar consideration would apply to the implication of neurofilaments or neurotubules, or both, in axonal transport (*4*). To investigate this possibility the L7 ganglia on each side of a series of four animals were injected with 5 to 10 μl of either [³H]-leucine or [³H]-lysine in saline solution at a concentration of 5 mc/ml (*1, 2*). The animals were subsequently killed. The nerve, ganglion, and dorsal root of one side was removed for determination of the distribution of activity. The nerve on the opposite side was left *in situ* in the dead animal

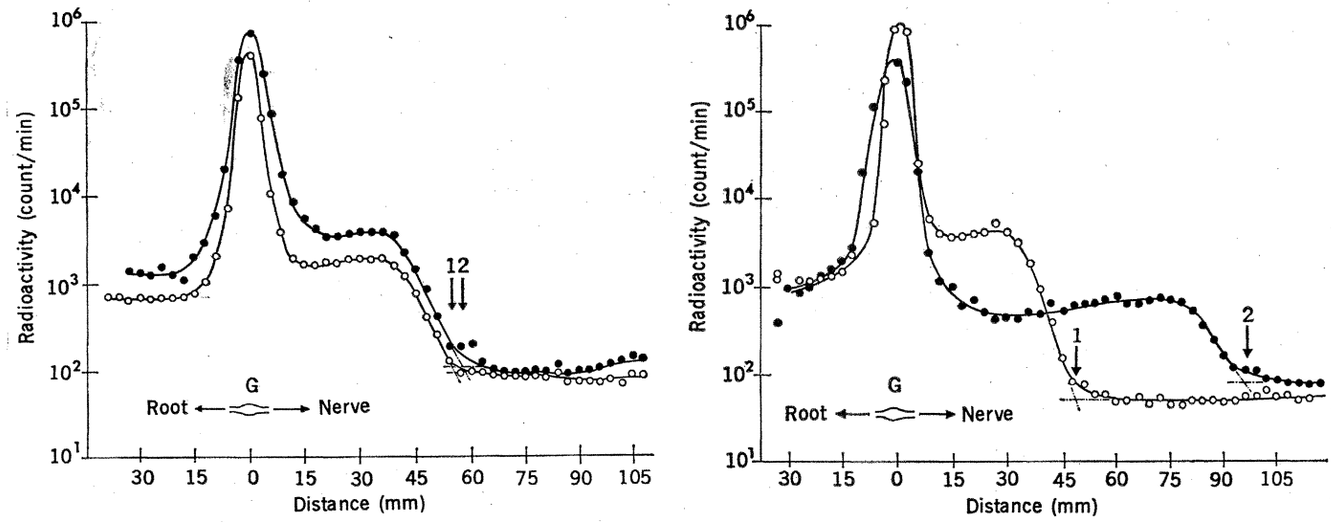

Fig. 1 (left). The two L7 ganglia were each injected with 7 μl of [³H]-leucine in saline solution (5 mc/ml). Three hours later the sciatic nerve, ganglion and dorsal root, from the left (○) side was removed from the animal and sectioned into 3-mm segments at the points represented along the abscissa. The radioactivity (counts/min) present in these segments is given by the log scale along the ordinate. The extent of transport is determined by the intersection of the slope of the forward edge of the crest with the base-line activity, shown at the foot of the crest by the dashed lines and arrow 1. The nerve on the right side (●) was left in situ in the dead animal for an additional 3 hours, and the small additional extent of the crest movement is shown by arrow 2. Fig. 2 (right). The L7 ganglia were injected with [³H]-lysine and the animal was killed 3 hours later. The control nerve, L7 ganglion and dorsal root, removed from the right side (○) shows a crest with the distal displacement shown by arrow 1 as expected of a fast transport lasting 3 hours. The opposite nerve (●) was placed in a chamber containing oxygen and kept moist with Ringer solution at 38°C for an additional 3 hours. The displacement of the crest in this preparation shown by arrow 2 is in accord with a fast axoplasmic transport for a period of 6 hours.