Table 1. Effect of varying insulin concentrations on glucose uptake by subcellular particles. Experiments were performed as in Fig. 1 except that whole cells were exposed to varying insulin concentrations before disruption. Data for one time point are shown, and only net or "specific" uptake is recorded—that is, D-glucose minus L-glucose uptake. The differences observed are all significant ($P < .05$).

Insulin (μunit/ml)	Glucose uptake (nmole/mg protein)	
	Control	Insulin
100,000	1.91	5.13
1,000	3.20	5.29
100	2.09	3.57
10	2.43	4.37

increase in D-glucose uptake when compared to controls (Fig. 1). There was essentially no change in the (lower) rate of L-glucose uptake by the membranes. The response to insulin was seen when the cells were exposed to concentrations as low as 10 μunit/ml (Table 1), within the physiologic range and near the lowest concentrations at which responses can be detected in isolated intact cells (*3*). Response to 1 μunit/ml was variable.

The effect of insulin in these experiments was entirely on glucose transport into the microsomal particles. The membrane preparations from both control cells and those treated with insulin were unable to convert D-[^{14}C]-glucose to either ^{14}C-CO_2 or ^{14}C-labeled lipid. Furthermore, the radioactive sugar taken up by the particles was quantitatively eluted with distilled H_2O and was recovered entirely as the unphosphorylated glucose. Thus all of the insulin effect was on the rate of D-glucose uptake and not on any subsequent step in glucose metabolism.

Insulin can be inactivated by alkaline hydroylsis. Such a preparation was tested against whole fat cells and showed no significant stimulation of D-[^{14}C]-glucose conversion to ^{14}C-CO_2. Membranes prepared from adipose cells exposed to this inactivated insulin preparation showed no significant increase in D-glucose uptake when compared to membranes prepared from control cells.

Addition of high concentrations of insulin (100 munit/ml) to the subcellular preparations after the adipose cells were disrupted led to no stimulation in the rate of glucose uptake. This was not unexpected. Rodbell (*4*) has shown that carefully prepared "ghosts" of adipose tissue cells still respond to insulin but that the response is far less than in intact cells even though the "ghosts" are able to metabolize glucose. Thus, there was no reason to suppose that metabolically inert particles prepared after more stringent treatment of the whole cells should respond to the hormone.

It has been demonstrated with other cell types that the usual "microsomal" fraction contains particles of plasma membrane (*5*), and it seems likely that the glucose uptake system responsive to insulin demonstrated here is in these fragments.

Whatever change in the membrane-bound glucose transport system is affected by insulin apparently requires the presence of intact cells at the time of exposure to the hormone but is retained after disruption of the cells. Whether this is because of a specific membrane configuration required for insulin binding that is lost upon disruption or because of a required "soluble" component that is lost during separation and centrifugation is not clear. However, the fact that the isolated membrane preparation retains the effect of insulin stimulation indicates that the hormonally induced change is reasonably stable and should be amenable to study in this isolated preparation.

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Dextranase Activity in Coleoptiles of Avena

Abstract. *An enzyme activity similar to that of dextranase is associated with coleoptiles of* Avena sativa. *When subjected to purified dextranase, both the pure natural dextran and the cell walls of the* Avena *coleoptiles yield isomaltose and isomaltotriose. Thus, the cell walls contain dextrans or dextran-like compounds. Coleoptiles with low auxin content have a lower dextranase activity than coleoptiles with high auxin content. The enzyme activity is therefore in some way sensitive to the hormone.*

The occurrence of β-glucanases in coleoptiles of *Avena* has been reported (*1*). As part of a study of α-glucanases, I now report the presence of an enzyme activity very similar to that of dextranase in these coleoptiles. Dextranases break down α-glucans in which the glycosidic linkages consist of $\alpha(1,6)$ and, in smaller quantiy, $\alpha(1,3)$ bonds. Until now no such enzyme activity has been reported in higher plants (*2*).

As a specific substrate for demonstrating dextranase activity, I used a natural dextran produced by *Leuconostoc mesenteroides* NRRL B-512. This dextran has high purity, has a molecular weight of about 20 to 30 million, and contains 95 percent of $\alpha(1,6)$ linkages and 5 percent of $\alpha(1,3)$ linkages. Some of these $\alpha(1,3)$ linkages form the branching points of long side chains (*3*). In addition, I used a commercial

Table 1. Dextranase activity in pellets prepared from 20 sections of coleoptiles of intact and decapitated *Avena* plants as judged by viscosity of dextran solutions after 4 hours of digestion. Activity expressed as flow seconds. Numbers in parentheses represent percent decrease in flow time as compared with control substrate.

Type of pellet	Flow time of 2% dextran (m. w. 20 to 30 $\times$ 10^6)				Flow time of	
	Exp. 1 (sec)	Exp. 2 (sec)	Exp. 3 (sec)	Exp. 4 (sec)	2% dextran (m. w. 2×10^6) (sec)	4% dextran (m. w. 2×10^6) (sec)
None	447	450	444	460	475	260
Boiled	447					
From intact coleoptile	404 (9.5)	369 (10.0)	376 (15.5)	390 (15.2)	433 (9.2)	236 (13.1)
From decapitated coleoptile	445 (0.0)	423 (6.0)			475 (0.0)	247 (5.0)

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