Plant Physiol. (1970) 45, 507–509

Short Communication

Carbowax 6000 Compared with Mannitol as a Suppressant of Cucumber Hypocotyl Elongation

Received for publication November 28, 1969

BURLYN E. MICHEL
Department of Botany, University of Georgia, Athens, Georgia 30601

Although some preparations of polyethylene glycol may contain toxic metal ions (6, 14), others apparently do not (3, 17). Toxic effects may not be restricted to dialyzable impurities (22) and may form by degradation (6). Injury or other aberrant responses have been reported without suggestion of cause (7, 15, 16). Other reports state or imply no toxicity, either from contaminants or the PEG[1] itself (1, 2, 9–13, 21–23, 25). Solutions of

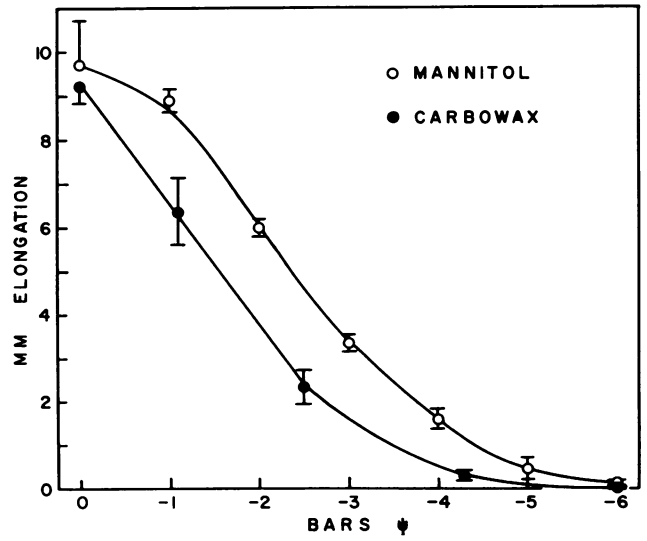

FIG. 1. Effects of decreasing water potentials from additions of Carbowax 6000 or mannitol on cucumber hypocotyl section elongation in a solution of IAA + GA_3 + $CoCl_2$ + KCl. Bars at data points indicate 2 × standard errors. Replications were 10.

one PEG, Carbowax 6000, have both produced injury (9, 14) and not (5, 17, 19). Carbowax 6000 was selected for this study because it does not pass the artificial membrane used and is less likely than the lower molecular weight forms to be absorbed by plants or plant cells.

The separation of osmotic from possible toxic effects of an osmoticum is not easy. It would seem best accomplished by comparison with an inert standard to which cells are impermeable. Mannitol has been used widely as being close to ideal; however, caution in its use is suggested (8, 17, 24). Although chosen for comparison with Carbowax 6000, some of the results indicate that mannitol may not have been entirely satisfactory.

[1] Abbreviation: PEG: polyethylene glycol.

MATERIALS AND METHODS

Asgrow Vigorpak cucumber (*Cucumis sativus* L. Marketer) seed was planted in polystyrene trays of sand and grown 4.5 days in a darkroom at 25 C. Under red light and beginning 1.5 mm below the top of the hypocotyl hook, ten 10-mm sections were cut with a tool (18) directly into 6 ml of solution in each 15- × 60-mm Petri dish. Treatment sequence was randomized. Unbuffered solutions (near pH 6) combining 10^{-6} M indoleacetic acid, 10^{-4} M gibberellin A_3, 10^{-4} M $CoCl_2$, and 10^{-2} M KCl, final strength, were used to stimulate elongation. Final section length was measured 24 hr after cutting, unless otherwise noted.

The water potential (ψ) values for mannitol are those of Morse (20). Through the courtesy of Dr. H. D. Barrs and with his thermocouple psychrometers, these were confirmed and values for Carbowax 6000 were obtained. Pharmaceutical grade Carbowax 6000 was purchased from Union Carbide Corporation. To check for possible dialyzable contaminants, two 30-ml samples of Carbowax solution (196 g/liter) were placed in closed, rigid osmometers.

Table I. *Elongation of Hypocotyl Sections Exposed to Untreated, Dialyzed, and Heated Carbowax and Concentrated Carbowax Dialysate*

Treatment	Elongation	
	No additive	With IAA + GA_3 + $CoCl_2$ + KCl
	mm	
Untreated Carbowax, made to −4.3 bars (163 g/liter)	0.3 bc[1]	0.6 c
Dialyzed Carbowax, 2 changes, 18 hr total, made to −4.3 bars	0.4 b	0.4 d
Dialyzed Carbowax, 3 changes, 20 hr total, amde to −4.3 bars	0.1 d	0.3 d
Heated Carbowax, 22 hr at 105 C, made to −4.3 bars	0.2 cd	0.3 d
Water	2.2 a	9.5 ab
Concentrated Carbowax dialysate equivalent to 163 g of Carbowax/liter	2.0 a	10.3 a
Concentrated Carbowax dialysate equivalent to 306 g of Carbowax/liter	...	8.8 b

[1] Values in the same column with the same letter are not significantly different at the 1% level in Duncan's new multiple range test. Replications were 20, first column, and 30, second column.

On each a stainless steel screen supported 30 cm^2 of U Zephyr membrane (Visking Co.) on which $Cu_2Fe(CN)_6$ had been precipitated. The samples were dialyzed against water (150-ml volumes, changed two and three times) for 18 and 20 hr. Both solution and water were continuously stirred with magnetic bars. The dialyzed solutions were made to −4.3 bars (163 g/liter). The dialysate was heated to reduce the volume to that equivalent to dialyzed Carbowax of 163 and 306 g/liter. To determine whether or not possible contaminants were heat-labile, 20 g of Carbowax were heated for 22 hr at 105 C (losing 66 mg) before testing.

Significance of elongation differences was tested at the 1% level with Duncan's new multiple range test (4). Elongation data were evaluated as ln (length − 8.5 mm) because this transformation made variance uniform over the range of elongation.

RESULTS AND DISCUSSION

Figure 1 shows the responses to various concentrations of mannitol and Carbowax obtained from sections in growth stimulants. The greater inhibition by Carbowax might be ascribed to toxicity. Any toxic materials present were not separable by dialysis (Table I). There was neither significant increase in elongation in dialyzed Carbowax solution nor inhibition from the concentrated dialysate. Results similar to those of Figure 1 were reported for *Avena* coleoptile sections by Jackson (7), who found no evidence of toxicity from Carbowax solutions to cell membranes of beet roots although grass seedling root hairs seemed to respond abnormally.

With section growth and respiration responding differently to many inhibitors, any toxic property of Carbowax might elicit differences in respiration in concentrations of Carbowax and mannitol producing similar growth effects. This did not occur (Fig. 2). Both levels of mannitol and Carbowax reduced respiration much less than growth—about 25 *versus* 50 and 40 *versus* 95%. Similar results were found for *Avena* coleoptile sections in mannitol (21). The inhibition of respiration was nearly constant from 7 hr on (Fig. 2), while the inhibition of growth was greater at 8 than at 24 hr (Table II). Toxicity is not indicated.

If toxicity were partially responsible for the reduction in elongation by Carbowax, injury might be transient or permanent. Recovery upon transfer from Carbowax and mannitol was tested (Table II). For concentrations suppressing growth equally, recovery from mannitol was significantly less than from Carbowax. Rapid recovery from brief exposure to Carbowax toxicity could be implicated; however, the reduced recovery (significant at 5% level) from −2.5 bar mannitol as compared with −4.3 bar Carbowax cannot be explained the same way. Exposure to low ψ could hardly account entirely for these lingering effects from mannitol.

The cell wall and exterior surface of the plasmalemma should be accessible to both solutes. Direct effects here, not associated with ψ, may be involved. Penetration of the plasmalemma by Carbowax or in appreciable amounts by mannitol is unlikely, but was not checked. The speed of aging, particularly related to changes in wall structure, also might be affected.

Carbowax 6000 produced greater inhibition of elongation at given values of ψ than mannitol; however, both may have inhibitory effects not entirely attributable to reduced ψ.

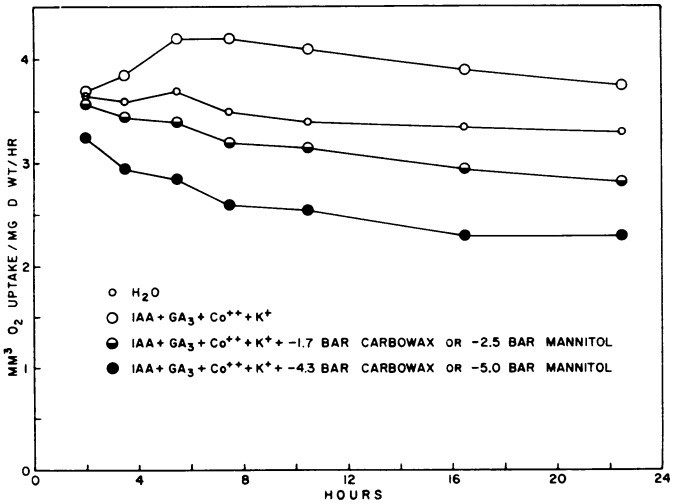

FIG. 2. Effects of growth stimulants and growth stimulants plus Carbowax 6000 or mannitol sufficient to reduce elongation by about 50% (−1.7 bar Carbowax and −2.5 bar mannitol) or 95% (−4.3 bar Carbowax and −5.0 bar mannitol) on rates of oxygen uptake of cucumber hypocotyl sections. Although individual replicates varied more, mean values for concentrations of Carbowax and mannitol reducing growth equally did not differ more than 0.1 unit; so they were averaged and plotted as single points.

Table II. *Total Elongation of Hypocotyl Sections Exposed to Carbowax or Mannitol for the Entire Growth Period or the First Portion Only*

Hours[1]	Elongation after Treatment in Addition to IAA + GA_3 + $CoCl_2$ + KCl				
	None	−1.7 bars Carbowax	−2.5 bars mannitol	−4.3 bars Carbowax	−5.0 bars mannitol
	mm				
4 + 0	3.0 b[2]	0.9 e	1.1 d	0.2 f	0.1 g
8 + 0	5.2 a	1.8 c	1.9 c	0.2 f	0.1 g
24 + 0	9.6 a	5.6 e	4.7 e	0.9 f	0.7 f
4 + 20	...	9.0 abc	8.1 bc	9.2 ab	7.8 c
8 + 16	...	8.6 abc	6.5 d	7.8 cd	4.9 e

[1] First figure indicates time in solution ± osmotic agent before transferring. Second figure indicates additional time in solution of growth stimulants only.

[2] Values within first two lines or within last three lines with the same letter are not significantly different at the 1% level in Duncan's new multiple range test. Values were separated into two groups because variance was less after a short than a long growth period. Replications were 30.

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