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Differences between Effects of Undissociated and Anionic 2,4-Dinitrophenol on Permeability of Barley Roots

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ABSTRACT

Effects of 2,4-dinitrophenol (DNP) and several other substituted phenols on permeability of barley roots (*Hordeum vulgare* var. Trebi) to ions were assayed as a function of pH and phenol concentration. Solutions containing 0.1 micromolar undissociated DNP increase the permeability of barley root cells to small ions such as K^+, Na^+, Ca^{2+}, and Cl^- with no inhibition of respiration. Undissociated forms of the other phenols increase permeability also, but they are less effective than DNP. Only the undissociated DNP is effective. Anionic DNP does not increase permeability or inhibit ion uptake, although it is the major species accumulated by the roots, both at pH 5 and pH 7. At pH 7, in contrast to pH 5, 10 micromolar DNP has no effect on ion permeability of barley roots yet it uncouples oxidative phosphorylation of barley root mitochondria. This indicates that the all too common use of DNP as a test for active transport or involvement of ATP synthesis can be misleading.

DNP[1] is used often as an inhibitor to assess the extent to which a physiological process under study is "active" or involves ATP synthesis. DNP is well-known as an uncoupler of oxidative phosphorylation. However, it also increases cell membrane permeability to ions (4, 15, 18). The present work was undertaken to determine whether an inhibitor such as DNP is multifunctional. Inconsistency in the literature with respect to the concentration of DNP that is effective suggested that the effects of DNP on permeability may be those of the weak acid form. This is suggested also by effects on permeability of short chain aliphatic acids of which only the undissociated form is effective (14). Consequently, effects of DNP on the permeability of barley roots to ions were studied as a function of pH, concentration, and time. Effects of several other phenols were studied also, to assess the specificity of DNP effects on permeability. Uptake of ^{14}C-labeled DNP was measured for comparison with the effects.

MATERIALS AND METHODS

The roots were from 6-d-old seedlings of barley (*Hordeum vulgare* var. Trebi) which had been dark-grown in aerated 0.2 mM $CaSO_4$ with or without 0.1 mM KCl at 22 to 25°C at pH 5.6. Roots were excised and rinsed several times with demineralized H_2O just before the experiment. Roots of intact plants which were used for some experiments were likewise rinsed.

Details of procedures and conditions for the experiments and analyses have been described (13). Briefly, about 5 g excised roots or roots of about 50 intact seedlings were maintained in 4 L aerated solutions of salts with or without DNP at 23°C. Compositions of the treatment solutions are given in the tables and figures. Successive samples of 0.5-g roots or five plants were removed periodically for determination of DNP, K^+, Na^+, Ca^{2+}, and Cl^-. Duration of treatment ranged from 0 to 24 h. The pH was maintained during growth and treatment by periodic titration with an appropriate acid or base. K^+ and Na^+ contents of the roots and solutions were measured by flame photometry, Ca^{2+} was measured by atomic absorption photometry, and Cl^- was measured by conductimetric titration (14). For determination of DNP uptake, roots of intact plants were held in solutions of [^{14}C]DNP, after which they were promptly excised and rinsed. The ^{14}C content of the roots was measured by scintillation counting as described previously (12). Respiration was determined by measurements of O_2 uptake through use of Clark oxygen electrodes in a closed system. Half-gram samples of roots were held in 20 ml of a rapidly stirred solution to a 50% decrease in O_2 concentration, generally for 20 to 90 min. Buffering capacities were determined by titration of root homogenates to pH 2.5 with 0.05 N HCl or to pH 11.5 with 0.05 N KOH as described (14).

Rates of [^{14}C]DNP uptake and fluxes of the ions were calculated from the data of linear time course curves by the method of least squares. Ion fluxes were calculated from changes in the ion content of the roots as a function of time, usually over the first 15 or 20 min for the efflux rates and for as long as the rates were constant for influx. Rates of influx determined from the initial constant rates of Na^+ and Cl^- uptake are the same as the isotopic influxes (13). Rates of K^+ and Ca^{2+} loss from the roots to the external solution are likewise the same as isotopic effluxes because the volume of the external solution is large enough to prevent reabsorption of the ions released from the roots.

RESULTS AND DISCUSSION

Effects of DNP on the Na^+ and K^+ content of roots vary with the pH as shown by roots in 10 μM DNP with 10 mM NaCl (Fig. 1). Sodium salts were used in most experiments so that cation influx (Na^+) and efflux (K^+) could be measured simultaneously. At pH 5, where the concentration of undissociated DNP (DNP-H) is 0.8 μM (K_d = 0.11 mM), K^+ efflux is large, especially relative to the amount of Na^+ accumulated. Both influx and efflux of ions are increased immediately by DNP at pH 5 (Table I), and the effects are independent of whether the roots have high or low salt content. The rates of Cl^- and Na^+ influx into roots in the DNP solution at pH 5 are much greater than the rates into roots in NaCl solutions without DNP. However, accumulations are low, presumably because of increased efflux.

Presence of Ca^{2+} in solution with DNP does not prevent these effects of DNP (Fig. 1B; Table II), although 1 mM $CaSO_4$ reduces permeability sufficiently to slightly slow ion uptake as shown in Table II. Because of this, many of the salt solutions were without

[1] Abbreviations: DNP, 2,4-dinitrophenol; PD, electrical potential difference.

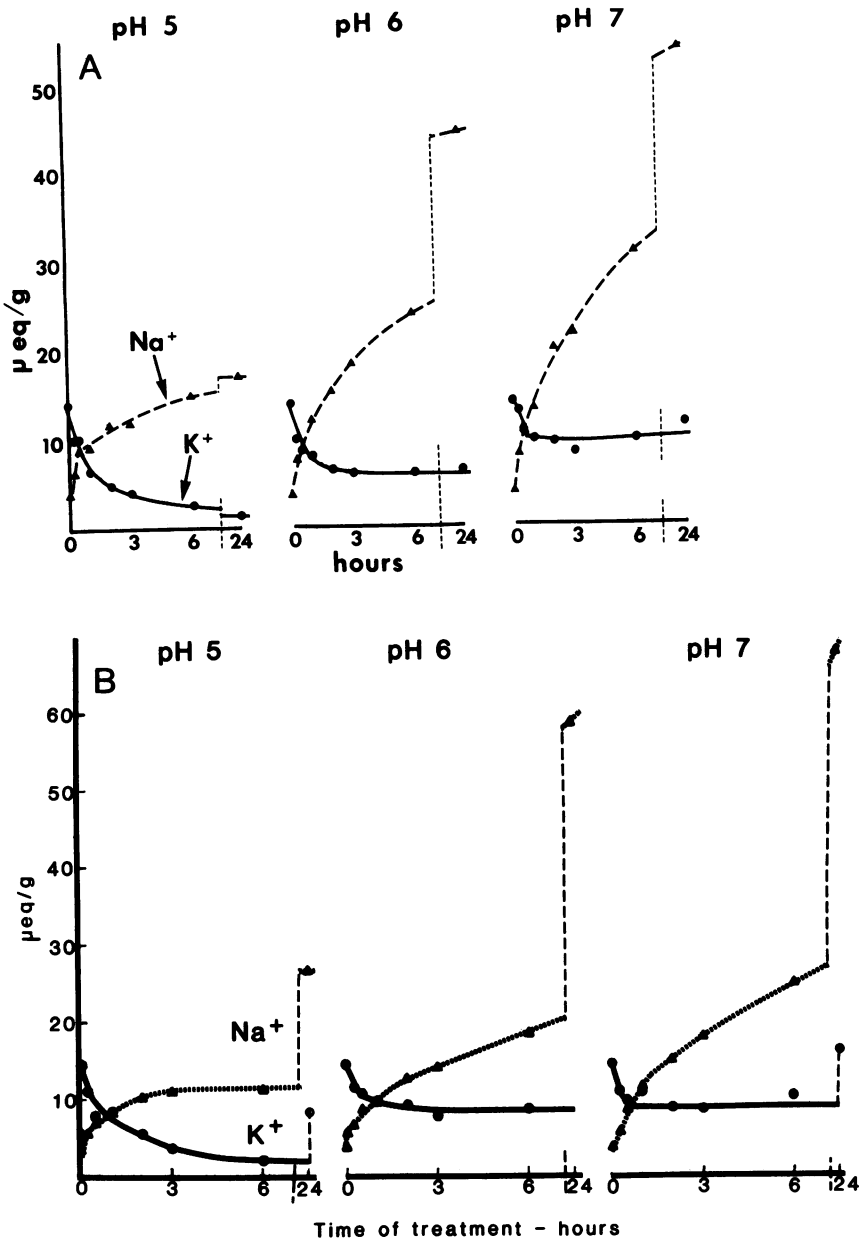

FIG. 1. Effects of 10 μM DNP on Na^+ and K^+ content of roots (μeq/g fresh weight) in 10 mM NaCl with (B) or without (A) 0.5 mM $CaCl_2$ as a function of pH and time of treatment.

Ca^{2+}. By contrast, K^+ efflux from roots in DNP at pH 7 is much less than at pH 5, although more Na^+ is accumulated (Fig. 1; Table I). Na^+ and Cl^- influx and accumulation and K^+ efflux are essentially the same at pH 7 as in the absence of DNP. Effects of DNP are intermediate at pH 6 where the concentration of DNP-H is 0.09 μM. These results indicate that 10 μM DNP at pH 5 increases the permeability of the roots to ions inasmuch as both influx and efflux of cations and anions are increased. This effect is consistent only with the concentration of DNP-H. It does not reflect the presence of anionic DNP, which is much higher in concentration than DNP-H.

Consistency with the DNP-H concentration was tested further by comparing the effect of varying the DNP concentration with the effect of varying the pH. Table III shows that a concentration of 1000 μM DNP is required at pH 7 to produce the same effect on permeability as 10 μM DNP at pH 5. The K^+ concentration was that required to balance the DNP concentration and adjust pH. It ranged from 0.1 to 1 mM at the highest DNP concentration. Comparative effects of 1 μM DNP at pH 5 and 100 μM DNP at pH 7 are likewise the same.

Changes in the effects of DNP with changes in pH might be expected if DNP was taken up at pH 5 but not at pH 7. But such is not the case (Fig. 2). More DNP is accumulated from 10 μM DNP with or without Ca^{2+} at pH 7 than at pH 5. The uptake, in contrast to the effects, is consistent with uptake of predominantly anionic DNP (DNP^-) which changes only slightly in concentration with a change in pH in this range. It is not at all consistent with the DNP-H concentration which decreases at pH 7 to 1.17% of the concentration at pH 5.

Roots in 10 μM DNP + 0.1 mM NaCl at pH 5 lose all of their endogenous Cl^-, 86% of their K^+, and 72% of their Ca^{2+} within 6 h with no accumulation of Na^+. The initial contents were: 5 μmol/g Cl^-, 17.8 μmol/g K^+, and 4.6 μeq/g Ca^{2+}. In spite of such large ion losses, respiration, as measured by O_2 uptake, is uninhibited (Table IV). Evidently, the increase in permeability is limited to relatively small ions inasmuch as buffering capacities are unaf-

Table I. *Effect of DNP on Ion Fluxes as a Function of pH*

Fluxes were measured over the first 15 to 30 min of treatment, during which time the rates were constant. The roots were maintained in 10 mM NaCl with or without 10 μM DNP. NaOH and HCl were used to adjust and maintain pH. Initial contents (μeq/g fresh weight) in low salt roots were Cl^-, 2.5; K^+, 12.9; Na^+, 5.3; and Ca^{2+}, 5.6. These values in high salt roots were Cl^-, 42.3; K^+, 72.6; Na^+, 7.8; and Ca^{2+}, 3.7.

Treatment	Flux Rates			
	Cl^-	K^+	Na^+	Ca^{2+}
	neq/min·g fresh wt			
Low salt roots				
10 mM NaCl				
+10 μM DNP	141	−16	202	
pH 5	177	−157	357	−43
pH 6	167	−67	252	−48
pH 7	127	NS	244	−18
High salt roots				
10 mM NaCl + 10 μM DNP				
pH 5	280	−176	327	−73
pH 6	218	−67	207	−73
pH 7	154	−14	266	−20

Table II. *Comparison of DNP Effects on Ion Content of Roots in the Presence and Absence of $CaSO_4$*

The solutions were all at pH 5 and contained the salts given in the first column.

Treatment	Cl^-	K^+	Na^+	Ca^{2+}
	μmol/g fresh wt			
Initial content	1.0	14.6	4.6	4.3
0.1 mM NaCl, pH 5				
3 h	4.7	14.1	12.3	2.9
6 h	11.1	15.2	17.0	3.0
24 h	19.8	16.4	26.9	3.5
+ 10 μM DNP				
3 h	0.2	6.5	4.6	2.8
6 h	0.1	2.8	3.5	2.6
24 h	0.3	1.0	3.3	2.5
0.1 mM NaCl + 1 mM $CaSO_4$				
3 h	6.8	14.3	11.1	4.3
6 h	7.8	13.7	13.8	4.3
24 h	15.3	14.4	24.5	4.8
+ 10 μM DNP				
3 h	0.1	7.4	4.7	4.7
6 h	0.1	4.1	3.6	4.4
24 h	1.8	3.8	4.5	4.1

fected. Some loss of buffering capacity (28%) occurs over the 24 h in 0.1 mM NaCl without DNP. This loss, which may be a decrease in respiratory substrates, may account for the decrease in respiration at 24 h. A respiratory decrease is usually observed over this time with the roots of 6-d-old barley plants under the conditions of the present work. Lack of respiratory inhibition in the presence of DNP, however, is consistent with its action as an uncoupler and indicates that the permeability increase is not caused by respiratory inhibition.

When roots have been in 10 μM DNP + 0.1 meq/L $CaSO_4$ for 3 h and then are placed in 0.1 mM KCl + 0.1 meq/L $CaSO_4$, rates of K^+ and Cl^- uptake recover to 121 and 78%, respectively, of the control rates (Table V). The roots had lost 70% of their endogenous K^+ and 86% of their endogenous Cl^- during the 3 h in DNP solution. Recovery is effective within the 15 to 20 s required to rinse and transfer the roots to the KCl solution (Fig. 3). No lag in the ion uptake is apparent. The rates of uptake are constant from this time over the 2-h period of measurement. DNP accumulated by the roots during the pretreatment remains with the roots without any effect on the ion fluxes or accumulation (Table V). Less than 3% is lost to the KCl solution.

Table III. *DNP Uptake and Effects on K^+ and Ca^{2+} as a Function of Undissociated DNP Concentration*

The treatment solutions contained DNP and KOH was used to balance the concentration of DNP^- and to adjust pH. The roots were treated for 6 h.

[DNP]	[DNP-H]	K^+	Ca^{2+}	DNP	DNP Uptake Rates
μM	*nM*	*μeq/g fresh wt*			*nmol/min·g*
None		17.1	4.5		
pH 5					
1	83	14.3	2.7	0.10	0.43
pH 7					
1	0.9	19.0	3.5	0.04	0.41
100	91	8.8	2.8	0.24	1.73
pH 5					
10	833	2.3	1.5	0.20	1.16
pH 7					
10	9.1	21.9	3.1	0.19	0.89
1000	908	4.9	1.6	0.38	8.03

Effects of several other substituted phenols were studied as a function of pH to assess the specificity of the effects of DNP-H. The phenols tested were: 2,4-xylenol (pK_a = 10.60); 2,6-xylenol (pK_a = 10.63); 2,5-dinitrophenol (pK_a = 5.22); 2,6-dinitrophenol (pK_a = 5.23); 2,4-chloronitrophenol (pK_a = 5.45); 2,4-nitrochlorophenol (pK_a = 6.46); 2,4-dichlorophenol (pK_a = 7.85); *p*-aminophenol (pK_a = 11.77); and 2,4-aminonitrophenol (pK_a = 8.0). Responses of high and of low salt roots to the most effective phenols are shown in Table VI, a and b. Like DNP, these phenols are the most effective when the concentrations of the undissociated forms are highest. Thus, high salt roots in the phenol solutions lose much more Cl^-, K^+, and Ca^{2+} than roots in water, at pH 5 but not at pH 7, where the concentrations of the undissociated phenol are less than 4% of the concentration at pH 5 (Table VIa). Low salt roots in 10 mM NaCl in the presence of the phenols at pH 5 also lose most of their K^+ and accumulate much less Na^+ and Cl^- than roots in 10 mM NaCl alone (Table VIb). The phenols have little or no effect on ion retention or accumulation at pH 7. Although these three phenols act much like DNP with the change in pH, they require 10-fold or higher concentrations to produce the same degree of effect as DNP. The other six phenols tested have no consistent effects on permeability at 0.1 mM, the highest concentration used, although the concentration of the undissociated form is as high or higher than the effective concentration of DNP-H. However, these phenols may require higher concentrations than 0.1 mM to produce the increase in permeability.

GENERAL DISCUSSION

Results of these studies clearly show that only DNP-H produces the increase in permeability of barley roots to ions. Neither presence nor uptake of anionic DNP has any effect on permeability. The most telling evidence for this is the data showing that 100 times the DNP concentration at pH 5 is required at pH 7 to produce the same effect as at pH 5 (Table III). The effects reflect only the concentration of DNP-H. Ineffectiveness of anionic DNP is further substantiated by lack of a difference between effects on high and low salt roots (Table I). Presence of high salt might be expected to compete with anionic DNP insofar as DNP competitively inhibits phosphate uptake by barley roots (10) and oxidative phosphorylation by barley root mitochondria (2, 11). Furthermore, DNP is about as effective in the presence of Ca^{2+} salt as in its

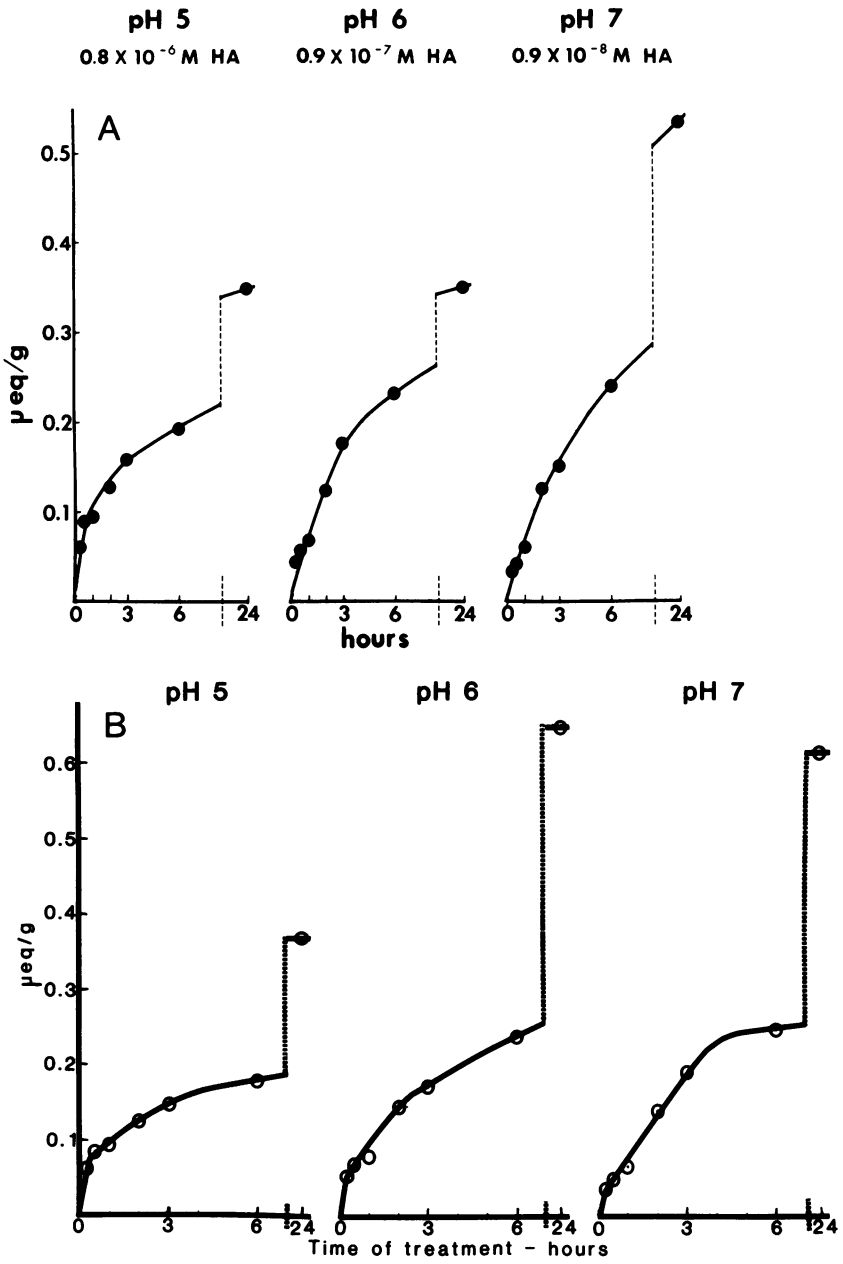

FIG. 2. Uptake of [^{14}C]DNP by roots in 10 μM DNP as a function of pH and time of treatment. The concentration of undissociated DNP (HA) is shown at each pH. The treatment solutions also contained 10 mM NaCl and, in B, 0.5 mM $CaCl_2$.

absence (Fig. 1B; Table II), although $CaSO_4$ is present at 100 times the DNP concentration. Insensitivity of the DNP effects to presence of salts in the external solution is another indication that DNP-H, not DNP^-, is the effective species.

The effects of DNP-H contrast greatly with the DNP uptake which involves predominantly DNP anions. This is shown by the large amount of DNP taken up at pH 7 compared to the uptake and effects at pH 5. A 10-fold increase in DNP concentration from 1 to 10 μM at pH 5 more than doubles the rate of DNP uptake (Table II). However, the pH change from 5 to 7 decreases uptake from 10 μM DNP only slightly. Thus, the DNP taken up at pH 5, as well as at pH 7, is predominantly anionic DNP.

Uptake of anionic DNP is no surprise considering its molecular size which is near that of other relatively small and hydrophilic organic anions taken up by barley roots (12). Barber and Koontz (3) attribute accumulation of DNP in the shoots of barley seedlings to the undissociated form because of greater accumulation at pH 4 than at pH 6. DNP in the roots was not measured. However, the likely permeability increase in the roots produced by 10 μM DNP at pH 4 could have contributed to the larger uptake at pH 4. This could be a factor in the somewhat faster initial rates of DNP uptake from 10 μM DNP at pH 5 in the present experiments. Borst-Pauwels (6) also attributes DNP uptake by yeast cells to only the undissociated form. However, his data are not consistent with uptake of only DNP-H. The data show that a 4-fold increase in DNP concentration (0.2 to 0.8 mM) at pH 4.5 doubles uptake, yet a 90-fold increase in the DNP-H concentration with a pH change from 7 to 5 does not even double uptake from 0.5 mM DNP. Thus, DNP effects, which reflect the concentration of DNP-H, and the uptake of DNP are not the same in tissues other than barley roots. Equating effects of DNP-H with DNP uptake, as is often done, can be erroneous.

The effect of DNP-H is only relatively specific. Three of the other substituted phenols tested likewise increase permeability, but at higher concentrations of the undissociated phenol than are required for DNP (Table VI). Although the experiments show

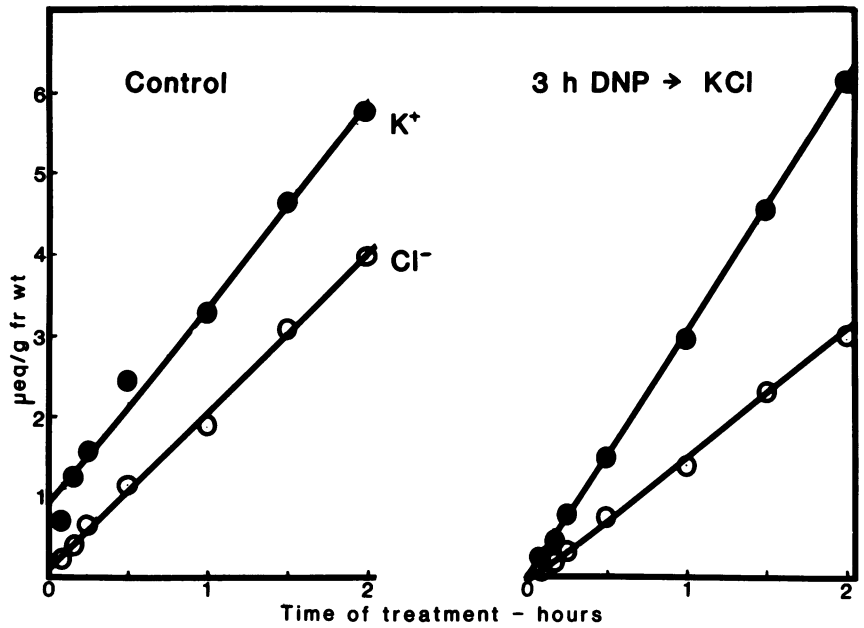

FIG. 3. Reversibility of undissociated DNP effects on K^+ and Cl^- uptake. The roots were pretreated for 3 h in 10 μM DNP (K^+ salt) + 0.1 meq/L $CaSO_4$ at pH 5 during which time they lost 70% of their K^+ and 86% of their Cl^-. They were then removed from the DNP solution, rinsed, and placed in 0.1 mM KCl + 0.1 meq/L $CaCl_2$ at pH 5. Transfer of the roots from the DNP solution to the salt solution without DNP took less than 20 s.

Table IV. *Effect on DNP on Buffering Capacity and Respiration of Barley Roots*

Treatment solutions were all at pH 5 and contained 0.1 mM NaCl with or without 10 μM DNP. A^- and HA indicate titratable anions (HCl titration) and acid (KOH titration), respectively.

Treatment	Buffering Capacity			O_2 Uptake Rate
	A^-	HA	$\sum(A^- + HA)$	
	μeq/g fresh wt			*neq/min·g*
Initial	86	67	153	784
0.1 mM NaCl				
3 h	72	63	135	736
6 h	65	54	119	640
24 h	57	53	110	408
				% control rate
+10 μM DNP				
3 h	70	63	133	101
6 h	64	59	133	105
24 h	64	65	129	112

Table V. *Reversibility of DNP Effects on Ion Accumulation*

Treatment solutions were all at pH 5 with the compositions shown under treatment. Roots in this experiment contained 17.7 μeq/g K^+ and 4.5 μeq/g Cl^- initially. The rates were constant from zero time for 2 or more h.

Treatment	Accumulation	
	Rate	0–2 h
	neq/min·g fresh wt	*μeq/g fresh wt*
0.1 mM KCl + 0.1 meq/L $CaCl_2$		
K^+	42	+5.7
Cl^-	32	+3.9
3 h, 10 μM DNP, K^+ salt		
DNP		0.307
2 h, 0.1 mM KCl + 0.1 meq/L $CaCl_2$		
DNP		−0.009
K^+	51	+6.1
Cl^-	25	+2.9

that the more lipophilic undissociated phenol is the effective form, relative effects of the phenols do not reflect their relative lipid solubilities as other investigators have found with other phenols (4, 9). The two xylenols (dimethylphenols), which are the least polar of the phenols tested, have no effects at the 0.1 mM concentrations used in this study. The other investigators cited have used concentrations of 0.25 to 5 mM, at pH 6 to 7.8. The relatively greater effectiveness of DNP compared to that of the other phenols used in these experiments may reflect a requirement for a somewhat amphoteric property, *i.e.* electronegative as well as electropositive. To wit, the more polar nitrophenols are more effective than the xylenols, aminophenols, and chlorophenols. Among the most effective phenols, either the *para* position of the nitro group or a balanced distribution of the polar nitro groups appears to be a factor. Thus, 2,4-chloronitrophenol is more effective than 2,4-nitrochlorophenol and effects of 2,4-dinitrophenol > 2,5-dinitrophenol > 2,6-dinitrophenol and 2,4-chloronitrophenol.

The effect of DNP on membrane permeability of barley roots has many features in common with the effects of other phenols and with the effects of DNP on phospholipid bilayer membranes. As in the present work, other phenols also increase barley root permeability to ions (3), indicating that such effects are not specific for DNP. Response to the phenols is immediate, as is the response of the roots to DNP (Fig. 1). The permeability effects are likewise rapidly reversible (4, 9). Barley roots also rapidly recover their ability to take up anions and cations after treatment with 10 μM DNP at pH 5 (Table V). The rapid response to the presence and absence of DNP and the nonspecificity of DNP observed in the present work and, in particular, the increase in the permeability of artificial membranes to K^+ and H^+ produced by DNP (5, 16) all suggest that the permeability effects of DNP-H are directly on the plasma membranes. Other investigators of roots, yeast, or neurons have come to a similar conclusion about the effects of DNP and other phenols on permeability (4, 9, 18).

The amount of DNP^- taken up by the roots at pH 7 is sufficient that, were it in free solution in the cytoplasm, the concentration of DNP-H formed at a cytoplasmic pH of 6 to 7 would be expected to increase membrane permeability internally. However, amounts

Table VIa. *Effects of Various Phenols on the Ion Content of High Salt Roots*

The solution contained 0.1 mM K^+ salts of the various phenols. Initial contents (μeq/g fresh weight) at pH 5 were Cl^-, 53.5; K^+, 89.4; and Ca^{2+}, 4.2.

Treatment	[Phenol-H]	pH 5			[Phenol-H]	pH 7		
		Cl^-	K^+	Ca^{2+}		Cl^-	K^+	Ca^{2+}
	μM	% initial content			μM	% initial content		
H_2O								
3 h		103	117	90				
6 h		90	101	85				
24 h		56	72	92				
0.1 mM 2-chloro-4-nitrophenol								
3 h	73.8	62	85	103	2.7	86	96	113
6 h		36	48	101		84	76	115
24 h		5	8	82		75	75	127
0.1 mM 2,5-dinitrophenol								
3 h	62.4	88	92	98	1.6	90	103	96
6 h		73	75	76		101	112	107
24 h		1.8	3.2	40		68	67	96
0.1 mM 2,6-dinitrophenol								
3 h	62.9	76	76	80	1.7	102	115	111
6 h		49	61	78		101	117	109
24 h		1.5	1.6	49		80	103	91

Table VIb. *Effects of Various Phenols on the Ion Content of Low Salt Roots*

The solutions contained K^+ salts of the various phenols. Initial contents (μeq/g fresh weight) at pH 5 were Cl^-, 7.0; K^+, 23; Na^+, 4.2; and Ca^{2+}, 5.8.

Treatment	pH 5					pH 7				
	[Phenol-H]	Cl^-	K^+	Na^+	Ca^{2+}	[Phenol-H]	Cl^-	K^+	Na^+	Ca^{2+}
	μM	μeq/g fresh wt				μM	μeq/g fresh wt			
10 mM NaCl										
3 h		40	20	56	3.9		40	22	60	4.5
6 h		51	19	72	4.2		59	22	86	4.6
24 h		71	18	89	4.0		76	23	109	4.6
+0.1 mM 2-chloro-4-nitrophenol										
3 h	73.8	5.6	5.2	15	2.8	2.7	7.1	11	18	4.5
6 h		4.9	3.0	12	2.6		13	7.2	28	4.8
24 h		32	3.8	39	2.1		71	40	103	5.1
+0.05 mM 2,5-dinitrophenol										
3 h	31.2	14	7.7	27		0.8	29	15	37	
6 h		18	5.3	33			35	8.8	51	
24 h		13	2.1	25			39	5.4	68	
+0.1 mM 2,6-dinitrophenol										
3 h	62.9	4.2	6.4	23	3.8	1.7	19	11	47	3.7
6 h		4.2	2.0	15	1.0		37	11	80	4.3
24 h		4.2	2.0	15	1.5		65	16	121	3.7

of free DNP in solution in the roots are not likely to be appreciable. Phenolics within tissues are known to be rapidly conjugated with sugars and other compounds. For example, all of the hydroquinine accumulated by barley roots was associated with glycoside and no free phenol could be found in the roots (8). Phenols can form dissociable charge-transfer complexes (7) and trinitrophenol can form simple ionic bonds with basic amino acids (17). However, phenols taken up by plants are not likely to be oxidized (1). Thus, most of the DNP accumulated in the barley roots is likely to be bound and very little is likely to be in free solution in the cytoplasm whether it is DNP^- or DNP-H.

Alteration of the permeability of the root membranes to ions sufficient to alter the imbalance of ion concentration across the cell membrane would be expected to alter the PD across the membrane. However, the kinetics of the PD changes produced by DNP and other phenols and the ion movements are not the same. For example, depolarization of the PD of excised barley roots produced by phenolic acids was complete with 12 min, but required 100 min to recover completely, although the rate of K^+ uptake recovered completely with 20 min (9). The PD of high salt maize roots exposed to 10 μM DNP was depolarized 23% in 15 min but thereafter gradually repolarized to at least as high as the

PD before addition of DNP, although exudation and exogenous K^+ and Cl^- transport to the sap were reduced during this time (19). DNP at 30 μM pH 5.9, had only a very slight effect on the intracellular PD of *Neurospora* (20). DNP concentrations of 1 mM produced a gradual change between −75 and −10 mv (20). In the present studies, the roots respond to the addition or removal of DNP immediately (Figs. 1–3). Thus, the ion fluxes apparently are not dependent on the magnitude of the PD.

Phenols are known to bind to proteins by H^+ bonding of the undissociated form of the phenol, thereby displacing protein-bound water and dehydrating the protein (for review, see Ref. 21). The binding is reversible. Presumably then, DNP-H effects on roots are owing to dehydration of membrane proteins involved in the permeability to hydrophilic ions.

DNP anions, which are the major species taken up by the roots, have no effect on ion uptake and retention, although DNP uncouples oxidative phosphorylation of barley root mitochondria (2, 11). The uncoupling action of DNP is competitive with Pi. This observation infers that anionic DNP is effective here. The effect of DNP on phosphate uptake by the roots, which is rate-limited by the phosphorylation (11), has been shown to act competitively as well (6). Thus, DNP appears to be bifunctional in accordance with its species. This suggests that use of DNP as a test for active transport or involvement of ATP synthesis can be misleading.

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