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THE EFFECT OF SOME OLIGO-AMINES AND -GUANIDINES ON MEMBRANE PERMEABILITY IN HIGHER PLANTS

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Key Word Index—*Beta vulgaris*; *Spinacia oleracea*; Chenopodiaceae; beet root; spinach; *Brassica napobrassica*; Cruciferae; swede; *Malus sylvestris*; Rosaceae; apple; membrane permeability; diamines; polyamines; guanidines; Synthalin; Guazatine.

Abstract—The effect of a series of oligo-amines and -guanidines on the membranes of higher plants has been tested by measuring the efflux of betacyanin from beet root discs, and the loss of total ions from beet root and swede discs, beet and spinach leaf discs and apple cells in suspension culture. All of the naturally occurring di- and polyamines tested showed relatively little toxicity. Betacyanin efflux from beet root discs was reduced by diamines [$NH_2(CH_2)_xNH_2$] up to $x = 10$ or less. Ion efflux was minimal at $x = 7$. Within the triamine series [$NH_2(CH_2)_xNH(CH_2)_3NH_2$] for $x = 8$ or less, betacyanin efflux was reduced or unaffected, although total ion loss was increased by the triamines when $x = 4$ or more and especially by the longer chain amines (to $x = 10$). Similar behaviour was found in the tetra-amine series [$NH_2(CH_2)_3NH(CH_2)_xNH(CH_2)_3NH_2$] with betacyanin efflux reduced for $x = 2$–4 (spermine). Although spermine potentiated the toxicity effects of Guazatine {[$NH_2C(=NH)NH(CH_2)_8]_2NH$} and Dodine [$NH_2C(=NH)NH(CH_2)_{11}Me$] in beet root discs, spermine and calcium ions reduced the ion efflux caused by these toxic guanidines and also by Synthalin B [$NH_2C(=NH)NH(CH_2)_{12}NHC(=NH)NH_2$] in swede discs, spinach leaves and apple cells. No significant reversal of ion loss was detected with putrescine, cadaverine or spermidine in swede discs. In the homologous series of monoguanidines [$NH_2C(=NH)NH(CH_2)_{x-1}Me$] for x up to 18, greatest toxicity was shown for $x = 10$ and 11 in both betacyanin loss and total ion efflux in beet root discs. Greatest ion efflux from the apple cell suspension was found with $x = 11$ and 12. In the homologous series of diguanidines [$NH_2C(=NH)NH(CH_2)_xNHC(=NH)NH_2$] for $x = 2$–12 greatest toxicity was shown for $x = 12$ (the longest chain tested) in beet root and in the efflux of ions from apple cell suspension. Technical Guazatine was considerably more phytotoxic than pure Guazatine in all systems tested. *p*-Chloromercuribenzoate (*p*-CMB) potentiates the loss of betacyanin and total ions caused by Guazatine, Synthalin B, and Dodine in beet root discs. This effect of *p*-CMB is reversed by subsequent incubation in cysteine or mercaptoethanol, prior to treatment with the guanidines.

INTRODUCTION

The polyamines spermine and spermidine which are ubiquitous in plants, animals and bacteria are thought to be involved in growth processes through interaction with nucleic acids. Recent experiments have indicated that these polyamines are also important for membrane structure. For instance, polyamines have been shown to stabilize protoplasts of oat leaf mesophyll cells [1–3] and of *Chlamydomonas reinhardii* [4]. Polyamines stabilize thylakoid membranes of barley chloroplasts and they prevent chlorophyll loss from barley leaf discs [5, 6]. They are also known to reduce efflux of betacyanin from beet root discs [7]. Furthermore, a recent patent claims that application of various di- and polyamines to crop plants can protect against frost damage, air pollution, loss of chlorophyll from cut vegetable crops and wilting [8].

It was of interest to study the effect of analogues of the polyamines on membrane integrity and the interaction of these with the naturally occurring polyamines. The effects of a series of guanidines have been studied, including the well-known fungicides Guazatine and Dodine. Guazatine protects against a wide range of cereal seed-borne diseases [9, 10], and it has been shown to have useful insect feeding repellancy [11, 12]. A homologous series of monoguanidines has been tested, including Dodine, a fungicide which is especially useful against spores of *Venturia inaequalis* [13]. A homologous series of diguanidines has also been investigated, including the deca- and dodeca-methylene compounds known as the Synthalins A and B respectively, which have been used as anti-diabetic drugs [14–16].

Membrane integrity has been studied by measurement of betacyanin efflux from beet tissue discs [7, 17, 18] and ion efflux from beet [17] and swede discs, beet and spinach leaf discs, and apple cell suspension cultures.

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RESULTS

Beet root discs were incubated with water or with 0.5 or 1 mM solutions of the compounds and betacyanin and total ion efflux measured after 1 hr. In the water controls the rate of betacyanin efflux was increased 3.1-fold and the rate of ion loss 2.4-fold on increasing the temperature from 20 to 37° (Fig. 1). All subsequent leakage experiments were conducted at 37°. In the control tissue betacyanin efflux and ion loss was rapid initially at both 20 and 37°. After 1 hr at 37° betacyanin efflux and ion efflux respectively were reduced to 20 and 16% of the initial value (Fig. 1). For the most toxic compound [guanidinoundecane (Table 1e)] betacyanin leakage (18× that of the control) represented almost complete depletion of the tissue in 1 hr. Ion leakage here was *ca* 80% of the total ions in the tissue released on boiling.

In the homologous series of diamines (Table 1a) $[NH_2(CH_2)_xNH_2]$ betacyanin efflux was markedly reduced with $x = 4$ (putrescine), less so when $x = 3, 5$ (cadaverine) or 6–8. Betacyanin efflux was hardly affected with $x = 10$, but an increased loss was found with $x = 12$. All diamines caused an increase in loss of total ions when tested at 1 mM. Loss was minimal and hardly significant when $x = 7$. The length of the spermidine molecule is almost identical to that of 1,8-diamino-octane ($x = 8$), and spermine is almost identical to that of 1,12-diaminododecane ($x = 12$). In the homologous series of triamines $[NH_2(CH_2)_xNH(CH_2)_3NH_2]$ (Table 1b) betacyanin efflux was reduced with $x = 3$ and 4 (spermidine) and marginally so for $x = 2$ and 8. Ion leakage increased progressively with increase in chain length and was considerable for $x = 10$. In the homologous series of tetra-amines $[NH_2(CH_2)_3NH(CH_2)_xNH(CH_2)_3NH_2]$ (Table 1c) for beet root discs treated with spermine ($x = 4$) betacyanin loss was reduced by almost half. Betacyanin loss was increased significantly only for $x = 10$. Total ion loss was hardly significant for $x = 2$, but it increased progressively with chain elongation. All of the methylated amines tested (Table 1d) showed a reduction of betacyanin efflux and *N,N,N'N'* - tetramethyl - 1,7 - diamino - 4 - azaheptane caused greatest reduction out of all the compounds tested. Moreover unlike spermine it did not cause increased ion efflux.

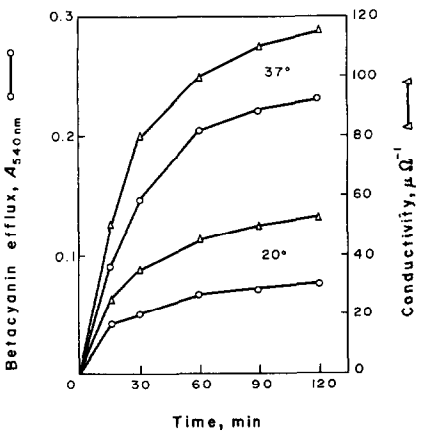


Fig. 1. Rate of betacyanin efflux (measured by spectrophotometry) and rate of ion loss (measured by conductivity) of beet root slices at 20 and 37°.

In the series of monoguanidines $[NH_2C(=NH)NH(CH_2)_{x-1}Me]$ (Table 1e) betacyanin and total ion efflux were hardly affected with $x = 6$ or less. Thereafter, toxicity increased abruptly with increased length of the aliphatic chain up to a maximum at $x = 10$ and 11. These were by far the two most toxic compounds tested. Beyond $x = 11$, activity was reduced with increasing chain length until with $x =$ 16–18 efflux was similar to the control values. Betacyanin efflux increased on lengthening the chain in the series of diguanidines $[NH_2C(=NH)NH(CH_2)_xNHC(=NH)NH_2]$ (Table 1f), up to $x = 12$ (Synthalin B, the longest chain tested), although, by comparison with the control, betacyanin loss was reduced for $x = 6$ and less. For $x = 8$ and 10, loss was similar to that found in the control. A considerable increase in betacyanin loss was found for $x = 12$. All diguanidines tested caused loss of total ions and this loss increased with chain length. In a collection of miscellaneous guanidines, hirudonine (Table 1g), like spermidine, its parent amine, caused a reduction of betacyanin efflux from beet root discs. Moreover, activities of putrescine, agmatine, arcain (Tables 1a, 1f and 1g) were similar, all giving a slight reduction of betacyanin efflux and a slight increase in total ion loss. Technical Guazatine was considerably more toxic than the purified material measured both in terms of betacyanin loss and ion efflux. The amine derived from Guazatine by hydrolysis (Table 1b) was somewhat less toxic than the parent compound. Streptomycin unexpectedly reduced betacyanin efflux though ion loss was slightly enhanced. MGBG had little effect on betacyanin efflux even at 1 mM though a slightly increased ion loss was observed. A preparation of the antifungal diguanidines, known as the hordatines [19, 20], extracted from the culms of barley seedlings was also tested. A solution containing *ca* 1 mM hordatine A/B, *ca* 2 mM hordatine M and *ca* 2 mM coumarylagmatine showed 95 and 116% of control for betacyanin efflux and ion loss respectively. A solution with a concentration five-fold greater gave 109 and 184% for the betacyanin efflux and ion loss respectively.

An experiment was conducted to investigate the interaction of spermine and Ca^{2+} with the toxic guanidines, Guazatine, Synthalin B and Dodine in beet root and swede discs. The results are shown in Table 2. Despite the reduction of betacyanin efflux found in the presence of spermine along (Tables 1c and 2), in combination with the Guazatine and Dodine, spermine increased the loss of betacyanin, though Synthalin B toxicity was apparently reversed. Ca^{2+} at 1 mM, like spermine, also causes a reduction of betacyanin loss. Although Ca^{2+} reverses the toxic effect of Guazatine and Synthalin B, it appeared to enhance the effect of Dodine in causing betacyanin and total ion efflux. Mg^{2+} at 1 mM had no effect on betacyanin efflux but it increased ion loss by 35%. Mg^{2+} had no significant effect on the betacyanin and total ion efflux due to spermine, Guazatine or Synthalin B (results not shown).

Measured by ion efflux, Dodine was very toxic to the swede discs (Table 2), as already shown for beet root tissue (Tables 1 and 2). Again technical Guazatine was considerably more toxic than pure Guazatine. Unlike the beet root tissue, the inclusion of

spermine with technical Guazatine, Synthalin B or Dodine causes a significant loss of toxicity in swede. Putrescine, cadaverine or spermidine at 1 mM gave little change in ion efflux and showed no protection against the guanidines (results not shown). By comparison with the root discs, relatively little toxicity was shown by Guazatine, Synthalin or Dodine in the leaf system (Table 3), and reversal by spermine and Ca^{2+} was only observed with the spinach leaves. Beet and spinach leaf discs behaved in a fundamentally similar way, as might be expected since they are both members of the Chenopodiaceae.

Ion efflux from the apple cell suspension culture was doubled by 0.5 mM technical Guazatine and Synthalin A or B (Table 4). The reversal of ion efflux was greater with spermine than with Ca^{2+} treatments, unlike the root and leaf tissue. The diguanidines with $x = 5$ and 6 showed little toxicity, but unlike other tissues tested, guanidinospermine was relatively toxic to apple cells. In the homologous series of monoguanidines, the apple cells showed greatest ion efflux with the compound $x = 12$ (Dodine) (Fig. 2). By itself, 1 mM spermine increased the efflux of the control by *ca* 40%. However, ion efflux induced by the monoguanidines was considerably reduced in the presence of 1 mM spermine. With chain lengths of $x = 16$ or longer, addition of spermine caused a loss of ions at rates slower than in the water controls.

The sulphydryl reagent *p*-chloromercuribenzoate (*p*-CMB) at 0.5 mM induced an increase of only 53% in betacyanin efflux and only 10% in ion loss in beet root discs (Table 5). However *p*-CMB potentiates the toxicity of technical Guazatine, Synthalin B and Dodine, measured both by betacyanin efflux and ion loss. In a further experiment (Table 6) beet root discs were pre-incubated for 1 hr with or without 0.5 mM *p*-CMB, then after washing in water they were incubated in water, 1 mM cysteine or 1 mM mercaptoethanol, and the betacyanin and total ion efflux measured for 1 hr. Although cysteine and mercaptoethanol *per se* increased the loss of betacyanin and total ions after incubation in the absence of *p*-CMB, the relative toxicities of the diguanidines was unchanged in the presence of these sulphydryl compounds. Again the toxicities of the guanidines were potentiated by the *p*-CMB. However, in tissues pre-incubated with *p*-CMB the toxicities of the diguanidines were considerably reduced after subsequent incubation in cysteine or mercaptoethanol by comparison with tissues pre-incubated in the absence of these sulphydryl compounds. This suggests that *p*-CMB can be displaced from its site of action by these sulphydryl-containing compounds. Cysteine, mercaptoethanol and *p*-CMB had no effect on the ion efflux of swede discs at the concentrations used in the present experiment.

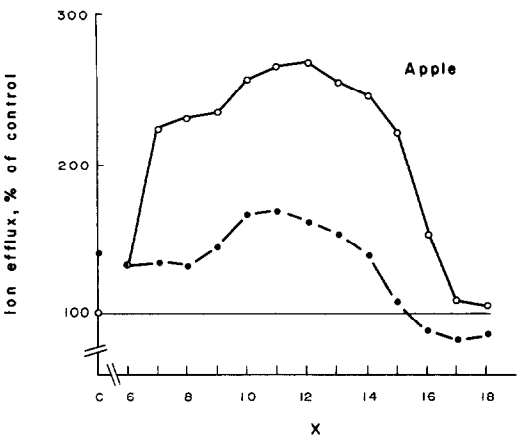


Fig. 2. Effect of a homologous series of aliphatic monoguanidines (0.5 mM) on the ion efflux from apple cell suspension culture in the absence (O) and presence (●) of spermine (1 mM). The ion efflux expressed as a percentage of the control after 1 hr immersion in the test solution is plotted against the length of the aliphatic chain, where x is the number of carbon atoms. The values for the control (c) are shown on the y-axis.

DISCUSSION

The present study indicates that certain aliphatic polyamines stabilize membranes and reduce loss of small molecules in a wide range of higher plant tissues. Other basic aliphatic compounds especially the guanidines, cause membrane disruption and an enhanced diffusional loss of metabolites. Although it is known that di- and polyamines interact with nucleic acids [21, 22], it seems unlikely that their stabilizing activity is mediated via nucleic acids in the present studies since their action on permeability is immediate. A direct interaction with membrane phospholipid seems a more likely explanation, as suggested also by the work of other investigators using plant systems [1, 5, 6] and bacteria [21, 23]. Spermine, spermidine and putrescine activate ATP-ase from *Vigna* mitochondria at less than 80 μM Mg^{2+} while the enzyme is membrane-bound but not when it is detached. Binding sites for the oligoamines are therefore probably present on the membrane [24].

Since the betacyanin is normally retained in the vacuole by the tonoplast in beet root [25], the differential effect of some of the amines (e.g. putrescine, spermidine and spermine) in causing reduction in betacyanin efflux and an increase in ion loss may suggest that these compounds preserve the tonoplast and disrupt the plasmalemma. Hypotheses invoking a single membrane with multiple sites for efflux appear to be less likely. Gibberellic acid and Ethrel also cause a decrease in betacyanin efflux but neither of these are as effective as spermine [18].

Antagonism of the toxic effects of basic drugs in micro-organisms by spermine is well established. The early work on this subject has been reviewed by Tabor and Tabor [23] and by Cohen [26]. The effect of spermine could be simulated by Mg^{2+} or Mn^{2+} but especially by Ca^{2+} ions in some of these studies. The effects of Ca^{2+} on membranes and in particular the stimulation of ion uptake in the presence of Ca^{2+} has been reviewed by Epstein [27].

The effects of polycations, e.g. spermine, in protecting membranes are not confined to damage by other cations as studied in the present work. For instance, spermine will also reverse membrane leakage induced by ethanol [7] and other amines are reported to reverse damage attributable to adverse conditions such as cold, wilting and pollution [8].

Table 1. Effect of amines and guanidines on betacyanin and total ion efflux from beet root discs

Name	Structure	% of control 0.5 mM Beta-cyanin efflux	0.5 mM Ion efflux	1.0 mM Beta-cyanin efflux	1.0 mM Ion efflux
(a) Diamines	$NH_2(CH_2)_xNH_2$				
1,3-Diaminopropane 2HCl	$x = 3$	87	121	80	155
1,4-Diaminobutane 2HCl (Putrescine)	$x = 4$	61	122	60	140
1,5-Diaminopentane 2HCl (Cadaverine)	$x = 5$	90	107	91	138
1,6-Diaminohexane 2HCl	$x = 6$	84	126	95	133
1,7-Diaminoheptane 2HCl	$x = 7$	87	88	95	106
1,8-Diaminooctane 2HCl	$x = 8$	88	110	88	130
1,10-Diaminodecane 2HCl	$x = 10$	90	117	102	138
1,12-Diaminododecane 2HCl	$x = 12$	148	138	180	176
(b) Triamines	$NH_2(CH_2)_xNH(CH_2)_3NH_2$				
1,6-Diamino-3-azahexane 3HCl	$x = 2$	98	103	96	101
1,7-Diamino-4-azaheptane 3HCl	$x = 3$	74	90	73	102
1,8-Diamino-4-azaoctane 3HCl (Spermidine)	$x = 4$	83	138	74	143
1,10-Diamino-4-azadecane 3HCl	$x = 6$	103	126	103	138
1,12-Diamino-4-azadodecane 3HCl	$x = 8$	85	124	85	140
1,14-Diamino-4-azatetradecane 3HCl	$x = 10$	115	168	124	242
1,17-Diamino-9-azaheptadecane 3HCl	$NH_2(CH_2)_8NH(CH_2)_8NH_2$	99	145	122	230
(c) Tetra-amines	$NH_2(CH_2)_3NH(CH_2)_xNH(CH_2)_3NH_2$				
1,10-Diamino-4,7-diazadecane 4HCl	$x = 2$	76	111	73	112
1,11-Diamino-4,8-diazaundecane 4HCl	$x = 3$	78	112	75	115
1,12-Diamino-4,9-diazadodecane 4HCl (Spermine)	$x = 4$	69	125	54	159
1,14-Diamino-4,11-diazatetradecane 4HCl	$x = 6$	100	135	109	177
1,16-Diamino-4,13-diazahexadecane 4HCl	$x = 8$	90	125	97	168
1,18-Diamino-4,15-diazaoctadecane 4HCl	$x = 10$	230	188	309	283

Table 1. (*continued*)

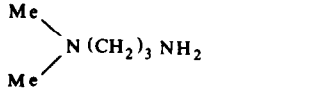
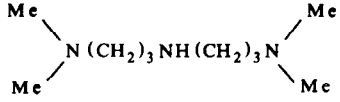
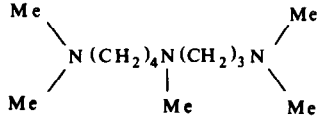
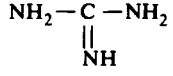
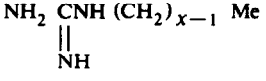
Name	Structure	% of control 0.5 mM Beta-cyanin efflux	0.5 mM Ion efflux	1.0 mM Beta-cyanin efflux	1.0 mM Ion efflux
(d) Methylated amines					
N,N-Dimethyl-1,3-diaminopropane 2HCl		65	128	58	151
N,N,N',N'-Tetramethyl-1,7-diamino-4-azaheptane 3HCl		65	101	52	99
N,N',N',N'',N''-Pentamethyl-1,8-diamino-4-azaoctane 3HCl		77	105	76	113
(e) Monoguanidines					
Guanidine HNO_3		134	107		
					
Guanidinomethane HNO_3	$x = 1$	109	136		
Guanidinoethane H_2SO_4	$x = 2$	121	113		
Guanidino *n*-hexane HOAc	$x = 6$	108	97		
Guanidino *n*-heptane HOAc	$x = 7$	149	121		
Guanidino *n*-octane HOAc	$x = 8$	420	173		
Guanidino *n*-nonane HOAc	$x = 9$	1318	728		
Guanidino *n*-decane HOAc	$x = 10$	1737	816		
Guanidino *n*-undecane HOAc	$x = 11$	1794	830		
Guanidino *n*-dodecane HOAc (Dodine)	$x = 12$	1263	634		
Guanidino *n*-tridecane HOAc	$x = 13$	1137	504		
Guanidino *n*-tetradecane HOAc	$x = 14$	608	256		
Guanidino *n*-pentadecane HOAc	$x = 15$	374	152		
Guanidino *n*-hexadecane HOAc	$x = 16$	197	131		
Guanidino *n*-heptadecane HOAc	$x = 17$	109	112		
Guanidino *n*-octadecane HOAc	$x = 18$	118	104		

Table 1. (*continued*)

Name	Structure	% of control 0.5 mM Beta-cyanin efflux	0.5 mM Ion efflux	1.0 mM Beta-cyanin efflux	1.0 mM Ion efflux
(f) Diguanidines	$NH_2CNH(CH_2)_xNHCNH_2$ (NH, NH)				
1,2-Diguanidinoethane 2HBr	$x = 2$	85	107	68	123
1,3-Diguanidinopropane 2HBr	$x = 3$	76	118	65	123
1,4-Diguanidinobutane H_2SO_4	$x = 4$	85	136	83	142
1,5-Diguanidinopentane 2HBr	$x = 5$	85	149	86	156
1,6-Diguanidinohexane 2HBr	$x = 6$	100	146	91	169
1,8-Diguanidinooctane 2HCl	$x = 8$	104	150	100	162
1,10-Diguanidinodecane 2HBr (Synthalin A)	$x = 10$	108	157	105	170
1,12-Diguanidinododecane 2HBr (Synthalin B)	$x = 12$	669	265	845	373
(g) Miscellaneous guanidines					
1, Guanidino-4-aminobutane H_2SO_4 (Agmatine)	$NH_2-C(=NH)-NH(CH_2)_4NH_2$	88	104	86	130
1,8-Diguanidino-4-azaoctane H_2SO_4 (Hirudonine)	$NH_2-C(=NH)-NH(CH_2)_4NH(CH_2)_3NH-C(=NH)-NH_2$	68	130	66	162
1,12-Diguanidino-4,9-diazadodecane 4HCl (Guanidinospermine)	$NH_2-C(=NH)-NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH-C(=NH)-NH_2$	85	141	74	185
1,17-Diguanidino-9-azaheptadecane 3HOAc (Guazatine)	$NH_2-C(=NH)-NH(CH_2)_8NH(CH_2)_8NH-C(=NH)-NH_2$				
(i) Technical		382	208	643	312
(ii) Pure		134	148	371	265

Table 1. (*continued*)

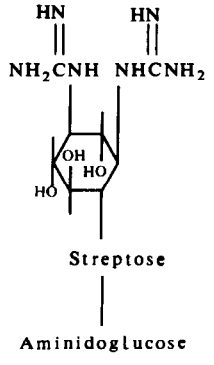
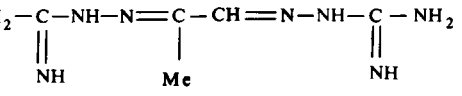
Name	Structure	% of control 0.5 mM Beta-cyanin efflux	0.5 mM Ion efflux	1.0 mM Beta-cyanin efflux	1.0 mM Ion efflux
Streptomycin H_2SO_4		68	142	66	182
Methylglyoxal bisguanyl hydrazone 2HCl (MGBG; Methylglyoxal bisamidino hydrazone)		102	138	104	165

Table 2. Effect of amines and guanidines on betacyanin and ion efflux from beet root discs and ion efflux from swede discs, and their antagonism by spermine and Ca^{2+}

		Beet root discs		Swede discs
	Concentration (mM)	Betacyanin efflux (%)	Ion efflux (%)	Ion efflux (%)
Control	—	100	100	100
Spermine	1.0	55	168	186
Guazatine (technical)	0.3	204	196	735
Guazatine (pure)	0.3	113	106	179
Synthalin B	0.3	338	375	1007
Dodine	0.3	520	300	1168
$CaCl_2$	1.0	55	146	167
$MgCl_2$	1.0	100	135	—
Spermine +	1.0			
Guazatine (technical)	0.3	355	356	526
Guazatine (pure)	0.3	122	116	148
Synthalin B	0.3	308	313	524
Dodine	0.3	750	433	756
$CaCl_2$	1.0	58	169	184
$CaCl_2$ +	1.0			
Guazatine(technical)	0.3	110	130	467
Guazatine(pure)	0.3	102	101	122
Synthalin B	0.3	126	147	489
Dodine	0.2	1240	600	713

Table 3. Effect of amines and guanidines on the efflux of ions from beet and spinach leaf discs and their antagonism by spermine and Ca^{2+}

	Beet leaves		Spinach leaves	
	Concentration (mM)	Ion efflux (%)	Concentration (mM)	Ion efflux (%)
Control	—	100	—	100
Spermine	1.0	168	1.0	156
Guazatine (technical)	0.5	350	0.3	157
Synthalin B	0.5	383	0.3	167
Dodine	0.5	332	0.3	163
$CaCl_2$	1.0	105	1.0	110
Spermine +	1.0		1.0	
Guazatine (technical)	0.5	361	0.3	123
Synthalin B	0.5	330	0.3	133
Dodine	0.5	325	0.3	133
$CaCl_2$	1.0	193	1.0	194
$CaCl_2$ +	1.0		1.0	
Guazatine(technical)	0.5	241	0.3	123
Synthalin B	0.5	203	0.3	138
Dodine	0.5	243	0.3	110

The diamines protect certain higher plants against the effects of several unrelated herbicides. Injury of Gramineae and Polygonaceae by *N* - (3,4 - dichlorophenylcarbamoyl) *N* - methylalanine was reduced by diamino-octane [28] and the injurious effects of the substituted benzoic acid Chloramben to soybeans were reduced by diaminodecane [29].

Amongst the compounds tested in the present work certain of the monoguanidines (Table 1e), including the fungicide Dodine, were the most toxic. In their studies on the effects of aliphatic monoguanidines on the germination of fungal spores, Byrde *et al.* [13] found considerable toxicity for $x = 11$–16 with optimum chain lengths varying slightly according to fungal species. In higher plants, a similar optimal chain length for toxicity was found, at $x = 11$ for beet root discs and $x = 11$ and 12 for apple cells grown in suspension culture.

Solapalmitine $\{[(Me)_2N(CH_2)_4]_2NC(=O)(CH_2)_{14}Me\}$ found naturally in *Solanum tripartitum* [30] and similar synthetic analogues caused leakage of *Escherichia coli* cell membranes at 10^{-5} M [31]. These compounds are structurally analogous to the aliphatic monoguanidines.

Out of a series of monoguanidines with $x = 2$–8, greatest inhibition of K^+ absorption by oat roots was found with the longest chain compound [32]. In a study of the effects of octylguanidine on the permeability of onion epidermal cells, Gomez-Lepe *et al.* [33] concluded that this monoguanidine acts primarily on the protoplast surface. On interaction with membrane proteins and phospholipids it behaved similarly to a surfactant.

Amongst the diguanidines tested (Table 1f), greatest betacyanin efflux was found on treatment of beet root discs with $x = 12$ (Synthalin B). In the trypanocidal activity of a homologous series of diguanidines from $x = 4$ to 18, maximum activity was found with $x = 10$–16 [34]. Moreover, the antibacterial activity of a homologous series of diguanidines was greatest with $x = 12$–18 [35].

It is of interest that the technical Guazatine is significantly more toxic in all biological systems tested than the pure Guazatine. This is despite the fact that Guazatine (1,17-diguanidino-9-azaheptadecane) is at a reduced concentration in the total solids of the technical Guazatine [36]. The greater toxicity of the technical Guazatine is probably due either to the presence of a compound other than Guazatine, or to a synergism between Guazatine and an impurity present in the technical sample. The triamine $[NH_2(CH_2)_8NH(CH_2)_8NH_2]$, formed by hydrolysis of pure Guazatine (Table 1b) and which might be expected in the technical sample, was considerably less toxic than the parent compound. Similarly diamino-octane (Table 1a) and diguanidino-octane (Table 1f) which might also be expected in the technical Guazatine were relatively non-toxic when tested in isolation.

Although guanidinospermine has the same molecular length as Synthalin B, unlike the latter it has relatively little activity in the beet root system (Table 1g). King *et al.* [34] found that diguanidino spermine was 1000-fold less effective than the Synthalins as a trypanocidal agent. Tomomatsu *et al.* [37], however, found that Synthalin A and diguanidino spermine were especially active as anti-tumour agents. Mihich *et al.* [38, 39] have also shown that Synthalin A inhibits the growth of leukemia.

Streptomycin (Table 1g) though an effective antibiotic and a compound known to interact with nucleic

Table 4. Effect of guazatine and a homologous series of diguanidines on the efflux of ions from apple cells grown in suspension culture and their antagonism by spermine and Ca^{2+}

	Concentration (mM)	Ion efflux (%)
Control	—	100
Spermine	1.0	153
Guanidinospermine	1.0	225
Guazatine (technical)	0.5	221
Diguanidinopentane	0.5	101
Diguanidinohexane	0.5	103
Synthalin A	0.5	206
Synthalin B	0.5	215
$CaCl_2$	1.0	144
Spermine +	1.0	
Guazatine (technical)	0.5	144
Synthalin B	0.5	146
$CaCl_2$	1.0	152
$CaCl_2$ +	1.0	
Guazatine(technical)	0.5	152
Synthalin B	0.5	162

Table 5. Effect of *p*-CMB, spermine and guanidines on the betacyanin and ion efflux from beet root discs

	Concentration (mM)	Betacyanin efflux (%)	Ion efflux (%)
Control	—	100	100
Spermine	1.0	57	157
p-CMB	0.25	150	115
	0.5	153	110
	1.0	163	100
Guazatine (technical)	0.2	130	116
	0.4	467	293
Guazatine (pure)	0.2	103	98
	0.4	153	129
Synthalin B	0.2	206	181
	0.4	587	444
Dodine	0.1	297	189
	0.2	560	318
Spermine +	1.0		
p-CMB	0.25	163	136
p-CMB	0.5	203	179
p-CMB +	0.5		
Guazatine (technical)	0.2	233	154
Guazatine (technical)	0.4	613	385
Guazatine (pure)	0.2	100	95
Guazatine (pure)	0.4	148	140
Synthalin B	0.2	773	405
Synthalin B	0.4	1473	722
Dodine	0.1	520	277
Dodine	0.2	800	426

Table 6. Effect of *p*-CMB, polyamines and guanidines on betacyanin and ion efflux from beet root discs

		Betacyanin efflux (%)						Ion efflux (%)					
		Water			*p*-CMB (0.5 mM)			Water			*p*-CMB (0.5 mM)		
	Concentration (mM)	Water	Cysteine (1 mM)	ME (1 mM)	Water	Cysteine (1 mM)	ME (1 mM)	Water	Cysteine (1 mM)	ME (1 mM)	Water	Cysteine (1 mM)	ME (1 mM)
Control*	—	100 (100)	100 (410)	100 (130)	100 (93)	100 (360)	100 (144)	100 (100)	100 (270)	100 (120)	100 (89)	100 (264)	100 (149)
Spermine	1.0	49	49	43	79	50	41	135	130	126	182	135	137
Guazatine (technical)	0.2	134	135	127	161	130	125	124	125	125	195	130	132
Guazatine (pure)	0.2	94	94	95	109	97	88	101	95	98	100	96	92
Synthalin B	0.2	221	219	208	381	236	165	186	189	175	362	200	186
Dodine	0.1	429	363	392	583	368	355	192	184	186	243	195	182

Beet discs were pre-incubated for 1 hr with or without *p*-CMB, then after washing in water they were again incubated with water, cysteine or mercaptoethanol (ME) for 1 hr. The discs were then again washed with water and immersed in the bathing medium containing various compounds to measure the efflux.

*The data in the vertical columns are normalized with reference to the treatment control (100%). The ratio of efflux for the various control treatments are given in brackets normalized with reference to the water control (100%).

acids, caused relatively little membrane leakage. Methylglyoxal bisguanylhydrazone known to interfere in polyamine metabolism and in the growth of tumours in animals [38–41] was also without effect on the beet root system.

The antifungal hordatines isolated from barley seedlings had little effect on the efflux of betacyanin or ions from the beet root discs. Experiments by Venis [42] suggest that the hordatines, like streptomycin, inhibit protein synthesis in pea stem segments and it is possible that this inhibition is the basis for the toxicity to fungi. Protein synthesis by the barley seedlings is apparently insensitive to hordatines. However, an increase in membrane permeability as the prime response may also consequently inhibit protein synthesis. The effect of the hordatines on membrane permeability of pea stem segments or fungal spores is at present unknown. However, the hordatines (having 16 carbons and 2 nitrogens as the molecular spacing between the two guanidino groups) were much less toxic to the higher plant system than the fungicide Guazatine, a compound which is structurally very similar, (having 16 carbons and 1 nitrogen).

Giaquinta [43] showed that sulphydryl groups are important for membrane function, since the non-permeant sulphydryl reagent *p*-chloromercuribenzenesulphonate inhibited membrane transport in leaf discs of beet, and Knauf and Rothstein [44] also concluded that sulphydryl groups are important for permeability in red blood cells. Naik and Srivastava [45] have also demonstrated an interaction of sulphydryl groups with polyamines in the regulation of membrane permeability.

The toxic effect of Guazatine, Synthalin B and Dodine was potentiated by *p*-chloromercuribenzoate, and reversal of this synergism by sulphydryl compounds is probably explained by the importance of sulphydryl groups for membrane integrity.

EXPERIMENTAL

Tissue discs. Beet root (*Beta vulgaris* L.) was grown locally, and swede (*Brassica napobrassica* Mill.) was obtained from a local shop. Discs (1 × 10 mm) were cut from the tissues and washed in tap H_2O for 1 hr (4 changes), and then in de-ionized H_2O for 30 min (2 changes). The discs were then blotted to remove surplus H_2O. Spinach (*Spinacia oleracea* L.) and beet leaf discs (diameter 10 mm) were used after washing once in H_2O. The tissue (10 root discs or 15 leaf discs) was immersed in 10 ml H_2O or test solns at 37°. Betacyanin efflux was measured by the increase in *A* at 540 nm. Total ion efflux was estimated by measuring the increase in conductivity with a Wayne Kerr Universal Bridge. Ion concn was measured at the start of the expt and after 1 hr immersion in the test solns.

Apple (*Malus sylvestris* Mill.) cv Miller's Seedling cell suspension cultures were grown in the dark in 150-ml flasks with 40 ml medium for 8 days on an orbital shaker at 30°, in principle by the method given in ref. [46]. The concn of the mineral salts and EDTA were as given in ref. [47]. Other components were (with final concn in brackets) nicotinic acid (0.5 mg/l.) pyridoxine–HCl (0.5 mg/l.), thiamine–HCl (0.1 mg/l.), inositol (100 mg/l.), 2,4-dichlorophenoxyacetic acid (2.5 μM), sucrose (20 g/l.) and asparagine (1 μg/l.). The cells were harvested by centrifugation and washed in 3% sucrose. Cells were resuspended (40% v/v) in 3% sucrose (primary suspension). For the ion efflux expts, 1 ml primary suspension (*ca* 15 mg dry wt.) was added to 9 ml H_2O or the test solns. After determining conductivity, the cells were incubated for 1 hr at 37° and conductivity then re-determined.

Chemicals. The homologous series of tri- and tetra-amines and methylated amines were donated by Dr. D. Brown. The synthesis and characterization of these is described in ref. [48]. The homologous series of diguanidines (apart from diguanidino-octane) was donated by Dr. W. G. Bardsley. Technical Guazatine was donated by Messrs. Murphy Co. Ltd., U.K. and pure Guazatine by KenoGard AB, Sweden. The hordatine preparation supplied by Mr. C. R. Bird was obtained from the culms of malted barley.

Diguanidinospermine (4HCl) and diguanidino-octane (2HCl) were synthesized from spermine and diamino-octane respectively with *O*-methylisouronium sulphate by the method of ref. [49]. The products were extracted into *n*-BuOH after addition of NaCl and NaOH, dried over dry Na_2SO_4 and the *n*-BuOH removed in a rotary evaporator. The products were dissolved in HCl and dried. Single Sakaguchi-positive, ninhydrin-negative spots were obtained on TLC (*n*-BuOH–HOAc–H_2O, 4:1:5; upper phase) on Whatman CC41 cellulose.

The triamine derived from Guazatine was prepared from 100 mg pure Guazatine by heating in 10 ml 10% NaOH at 120° under 1 atm pres. for 30 min [50]. After addition of 2 g NaCl the product was extracted with 2 × 40 ml *n*-BuOH. The organic layer was dried over Na_2SO_4, filtered, acidified with HCl and concd to dryness (yield *ca* 100%). The product was Sakaguchi-negative and ninhydrin-positive.

Other chemicals were purchased from Aldrich, BDH, Fluka and Sigma.

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