RESULTS

R E S U L T S

Development of peroxidase and ATPase activity in maize scutellum

Scutella were excised from seedlings germinated for different periods and assayed for peroxidase and ATPase in slices. The results reported in Table-1 show that peroxidase activity when assayed on the day of excission (0 day incubation) increased with germination upto 8 days and then remained constant. If the excised scutella were kept for incubation for different periods, the peroxidase activity increased continuously until it reached a maximum level (approx. 200 units/g tissue). The development of enzyme was more rapid in the scutella excised and incubated rather than in the freshly excised scutella from the seeds germinated for the same period.

Similar results were obtained with ATPase also (Table-2). ATPase activity increased upto 9th day and like peroxidase, the excised and incubated scutella showed a more rapid increase in the development of the enzyme than the freshly excised ones for the same period. Longer periods of incubation of scutella obtained from seedlings germinated for 8-10 days showed a slight decrease in ATPase activity.

TABLE-1 : Development of peroxidase activity in excised maize scutellum

Scutellum excised from seeds germinated for (days)	Peroxidase activity (units/g tissue) in excised scutellum incubated for (days)					
	0	1	2	3	4	5
1	5	17	77	92	145	156
2	25	92	135	150	160	172
3	61	97	137	163	184	190
4	77	116	140	173	186	196
5	112	143	163	176	185	195
6	131	151	173	182	197	201
7	140	162	175	196	201	202
8	148	162	182	196	202	201
9	151	173	198	201	202	200
10	152	173	190	200	202	201

Scutella were excised from seedlings germinated for different days starting from the day of transfer to Petridishes and incubated for upto 5 days. Enzyme activity was assayed in the slices.

TABLE-2 : Development of ATPase activity in excised maize scutellum

Scutellum excised from seeds germinated for (days)	ATPase activity (units/g tissue) in excised scutellum incubated for (days)					
	0	1	2	3	4	5
1	8	27	30	33	49	55
2	14	35	39	45	60	63
3	15	42	49	61	63	76
4	31	53	54	69	77	87
5	34	60	71	88	89	94
6	42	67	72	89	93	100
7	56	71	79	93	108	102
8	61	83	89	105	101	89
9	70	83	97	107	95	89
10	72	102	108	102	93	88

Growth and other conditions were same as given in Table-1.

Scutella from seedlings germinated for 4 days had a peroxidase activity of 77 units/g tissue when assayed in the slices, however, when assayed in homogenate the total activity was 300 units/g tissue showing that only 25% of the total activity has been assayed in slices. Later studies (Table-13) showed that membrane fraction (40,000g fraction) had 30% of the total activity present in the homogenate. These results suggested that only membrane bound peroxidase is assayed in slices. Similarly ATPase activity in slices (31 units/g tissue) was approximately 6% of the total activity present in the homogenate. Wheeler et al. (1979) have provided evidence that the enzyme assayed in maize scutellum slices is plasmalemma bound ATPase only. Unless otherwise stated scutella from seedlings germinated for 4 days were used for most of the studies described hereafter.

Effect of amines and guanidines on peroxidase and ATPase activity

The effect of a series of diamines and polyamines as well as guanidino compounds was tested by preincubation of slices from scutella of 4 or 8 day germinated seedlings with the compounds for 1 hr before assaying the enzyme activity. The results reported in Table-3 show that in the slices from 4 day germinated seedlings all the di- and

TABLE-3 : Effect of amines and guanidino compounds on peroxidase and ATPase in maize scutellum slices

Compound	Enzyme activity (units/g tissue) from seedlings germinated for (days)			
	Peroxidase		ATPase	
	4	8	4	8
--	75	150	29	56
1,2-Diaminoethane 2 HCl	66	147	27	56
1,3-Diaminopropane 2 HCl	50	150	28	56
1,4-Diaminobutane 2 HCl (Putrescine)	16	147	41	56
1,5-Diaminopentane 2 HCl (Cadaverine)	47	150	37	56
1,6-Diaminohexane 2 HCl	53	147	32	56
1,8-Diaminooctane 2 HCl	65	147	28	56
1,10-Diaminodecane 2 HCl	66	147	30	56
1,8-Diamino-4-azaoctane 3 HCl (Spermidine)	14	145	43	58
1,12-Diamino-4,9-diazadodecane 4 HCl (Spermine)	12	144	42	61
2-Amino-5-guanidino-n-valeric acid HCl (Arginine)	82	153	34	56
1-Guanidino-4-aminobutane H_4SO_4 (Agmatine)	78	151	33	56
N-Guanyl N-methyl glycine (Creatine)	68	150	31	56
2-Imino-N-methyl hydantoin (Creatinine)	76	150	29	54

contd.

TABLE-3 (Contd.)

Compound	Enzyme activity (units/g tissue) from seedlings germinated for (days)			
	Peroxidase		ATPase	
	4	8	4	8
N-Amidino-glycine (Guanidino-acetic acid)	104	156	40	56
4-Guanidino-ethyl acetic acid (Guanidino-butyric acid)	104	154	41	57
Guanidino-n-dodecane HOAc (Dodine)	105	157	46	61
1,17-Diguanidino-9-azaheptadecane 3HOAc (Guazatine)	109	157	45	60

Scutellum slices from 4 or 8 day germinated seedlings were incubated with the compounds (2 mM) for 1 hr. After incubation the slices were washed with water and used for enzyme assay.

polyamines inhibited peroxidase activity and the maximum inhibition was by spermidine and spermine. Among the diamines the inhibition increased with chain length upto diaminobutane (putrescine) which gave 80% inhibition and then decreased with further increase in chain length. In contrast to the inhibition of peroxidase, the ATPase activity was activated by putrescine and cadavarine as well as the two polyamines spermidine and spermine. The activation varied from 30-45% by these compounds. Out of the guanidino compounds tested arginine, agmatine, creatine and creatinine had no effect on peroxidase or ATPase activity but guanidino-acetic acid (GAA), guanidino-butyric acid (GBA) and the two guanidino fungicides dodine and guazatine showed about 40-60% activation of both peroxidase and ATPase activity. None of the compounds had any effect on either peroxidase or ATPase activity in the scutella excised from 8 day germinated seedlings.

These results indicated that with increase in period of germination from 4 to 8 day, which may result in disintegration and loss of membrane integrity, the effect of amines and guanidines was abolished. The compounds thus appear to effect peroxidase and ATPase activity by altering the membrane structure. This was further evident when homogenate instead of slices was used (Table-4). The compounds had some effect on the enzymes in the homogenate prepared from

TABLE-4 : Effect of amines and guanidino compounds on peroxidase and ATPase activity in maize scutellum homogenate

Compound	Enzyme activity (units/g tissue) from seedlings germinated for (days)			
	Peroxidase		ATPase	
	4	8	4	8
--	308	502	475	800
1,2-Diaminoethane 2 HCl	304	500	485	787
1,3-Diaminopropane 2 HCl	296	500	516	800
1,4-Diaminobutane 2 HCl (Putrescine)	286	494	573	813
1,5-Diaminopentane 2 HCl (Cadaverine)	289	498	547	787
1,6-Diaminohexane 2 HCl	298	496	501	787
1,8-Diaminooctane 2 HCl	298	500	490	800
1,10-Diaminodecane 2 HCl	304	496	485	800
1,8-Diamino-4-azaoctane 3 HCl (Spermidine)	240	492	625	813
1,12-Diamino-4,9-diazadodecane 4 HCl (Spermine)	258	494	645	813
2-Amino-5-guanidino-n-valeric acid HCl (Arginine)	324	502	511	787
1-Guanidino-4-aminobutane H_4SO_4 (Agmatine)	326	506	516	800
N-Guanyl N-methyl glycine (Creatine)	320	502	501	787
2-Imino-N-methyl hydantoin (Cratinine)	310	504	485	787

Contd..

TABLE-4 (Contd.)

Compound	Enzyme activity (units/g tissue) from seedlings germinated for (days)			
	Peroxidase		ATPase	
	4	8	4	8
N-Amidino glycine (Guanidino-acetic acid)	370	502	568	800
4-Guanidino-ethyl acetic acid (Guanidino-butyric acid)	366	504	583	817
Guanidino-n-dodecane HOAc (Dodine)	374	512	645	817
1,17-Diguanidino-9-azaheptadecane 3 HOAc (Guazatine)	382	514	655	800

Scutellum homogenate from 4 or 8 day germinated maize seedlings was incubated with compounds (1 mM) for 1 hr. To the homogenate assay components were added after 1 hr incubation and the enzyme activity was assayed.

scutella excised from 4 day germinated seedlings but had no effect on the homogenate prepared from scutella excised from 8 day germinated seedlings due to the breakdown of membrane structure during homogenization. The disintegration and loss of membrane integrity of the scutella during germination was further confirmed in a study where malondialdehyde (MDA) level, which is a measure of lipid peroxidation and an index of membrane integrity, was estimated. The results reported in Table-5 show that MDA content started increasing from 4th day onwards and by 8th day it was 4 fold higher than on the 1st day.

The compounds showing an effect on the two enzymes were then tested at different concentrations with slices and homogenate. The results reported in Table-6 show that in slices the inhibition of peroxidase by putrescine, spermidine and spermine increased with concentration and the activity was completely inhibited with 3 mM spermidine and spermine. With GAA and dodine, however, the activity increased upto 2-3 mM and then decreased to the control level with increasing concentrations. With homogenate, the inhibition by putrescine, spermidine and spermine and activation by GAA and dodine was considerably reduced. The guanidino compounds even showed some inhibition at higher concentrations. In case of ATPase (Table-7) polyamines and guanidines when tested at different concentrations showed activation upto 2 mM although it was more with slices compared to homogenates.

TABLE 5 : Lipid peroxidation in maize scutellum on different days of germination

Scutellum excised from seeds germinated for (days)	Malondialdehyde (nmol/g tissue)
1	30
2	26
3	27
4	38
5	43
6	52
7	74
8	127
9	135
10	139

TABLE-6 : Effect of concentration of polyamines and guanidines on peroxidase activity in maize scutellum slices and homogenate

Concentration (mM)	--	Peroxidase activity (units/g tissue) with Putrescine	Spermidine	Spermine	Guanidino acetic acid	Dodine
			Slices			
0	75					
0.5		62	32	43	86	85
1		50	19	22	94	102
2		28	13	12	104	105
3		18	5	2	91	110
4		11	2	2	73	96
5		11	2	2	71	80
			Homogenate			
0	305					
0.5		302	287	278	357	345
1		293	259	271	363	375
2		284	238	256	366	369
3		259	223	238	296	262
4		244	210	226	262	244
5		217	189	189	256	223

Contd.

TABLE-6 (Contd.)

Slices and homogenate were prepared from scutella of 4 day germinated seedlings. They were preincubated at 37° with the compounds at different concentration for 1 hr. Slices were then washed with water and enzyme activity was assayed. For enzyme activity test in the homogenate, other assay components were added after pre-incubation with amines and guanidines.

TABLE-7 : Effect of concentration of polyamines and guanidines on ATPase activity in maize scutellum slices and homogenate

Concentration (mM)	ATPase activity (units/g tissue) with				
--	Putrescine	Spermidine	Spermine	Guanidino acetic acid	Dodine
Slices					
0 28					
0.5	31	32	32	32	38
1	33	35	35	36	48
2	38	42	40	39	44
3	37	33	33	33	43
4	30	30	30	31	43
5	28	29	29	31	37
Homogenate					
0 475					
0.5	532	557	557	532	568
1	557	578	604	562	599
2	573	624	650	568	645
3	521	578	619	557	562
4	485	480	568	532	506
5	470	475	557	475	495

Conditions were same as given in Table-6.

Raising the concentration beyond 2 mM decreased the activation continuously in slices as well as in homogenates until it reached the control level at 5 mM, except with dodine where some activation was still evident in slices. The biphasic response of polyamines was not due to the displacement of Mg^{++} from Mg^{++}-ATP since the reversal of activating effect at high concentration of polyamines was not affected by increasing the Mg^{++} concentration (Table-8).

Interaction between polyamines and guanidines for peroxidase and ATPase activity

The question whether polyamines and guanidines interact for common binding sites on membrane to produce their effect on the two enzymes was studied by preincubating the slices with one compound followed by the other after washing and the activity was compared with a control group treated with water for the same period. The results reported in Table-9 for peroxidase activity show that when the preincubation was carried out with water first followed by different compounds, putrescine, spermidine and spermine showed inhibition but GAA and dodine showed activation. Arginine, agmatine, creatine and creatinine had no significant effect. However, if the slices were first incubated with putrescine, spermidine and spermine and then with guanidino compounds, the inhibitory effect of polyamines was completely abolished by all the guanidino compounds

TABLE-8 : Effect of Mg^{++} concentration on ATPase activity in spermine treated maize scutellum slices

Mg^{++} concentration (mM)	ATPase activity (units/g tissue)
10	28
20	29
30	29
40	28
50	30

Scutellum slices were preincubated in 5 mM spermine at 37° for 1 hr. Then the slices were washed and assayed for enzyme activity in presence of different concentrations of Mg^{++}.

TABLE 9 : Interaction between polyamines and guanidines for peroxidase activity in maize scutellum slices

Treatment Ist / 2nd	Peroxidase activity (units/g tissue)									
	Water	Putre-scine	Sper-midine	Sper-mine	Argi-mine	Agma-tine	Crea-tine	Creat-inine	Guanidino acetic acid	Dodine
Water	76									
Putrescine	28				93	93	84	28	110	125
Spermidine	16				91	87	84	16	108	119
Spermine	15				84	78	76	14	106	114
Arginine	90	91	87	93						
Agmatine	85	87	85	83						
Creatine	82	85	82	81						
Creatinine	78	30	18	16						
Guanidino acetic acid	118	106	106	102						
Dodine	124	116	106	103						

Contd.
2

TABLE-9 (Contd.)

Scutellum slices from 4-day germinated seedlings were preincubated (first treatment) for 1 hr in polyamines and guanidines (2 mM) at 37°. After washing in water, the polyamine-treated slices were again incubated in guanidines and guanidine-treated slices were incubated in amines (second treatment) for 1 hr. The slices after the second incubation were washed in water and used for enzyme assay. A control group incubated in water for the same period was included.

except creatinine. GAA and dodine still activated to almost same extent as without the amines pretreatment. Further, if the pretreatment was carried out first with the guanidino compounds and then with polyamines, the polyamines could not reverse the effect of guanidino compounds. These results suggest that the binding of polyamines was reversed by compounds having free guanidino groups but the guanidine binding was not reversed by polyamines. It is interesting to note that although arginine, agmatine and creatine had no significant effect by themselves, they prevented the effect of polyamines on peroxidase activity. Creatinine, which has no free guanidino group and had no effect by itself, was unable to prevent the inhibitory effect of polyamines.

A similar study on ATPase (Table-10) showed that incubation of slices in water followed by polyamines and guanidines resulted in the activation of the enzyme by all the polyamines, GAA and dodine. However, incubation with polyamines first and then with GAA and dodine or vice versa did not show any additive effect. Arginine, agmatine and creatine, which had no effect by themselves, considerably reduced the effect of polyamines whether the slices were incubated with these compounds first and then with polyamines or vice versa. Creatinine, which has no free guanidino group, could not abolish the effect of polyamines. Even though both polyamines and some of the guanidines

TABLE-10 : Interaction between polyamines and guanidines for ATPase activity in maize scutellum slices

Treatment 1st / 2nd	ATPase activity (units/g tissue)									
	Water	Putre-scine	Sper-midine	Sper-mine	Argi-nine	Agma tine	Crea-tine	Creat-inine	Guani-dino acetic acid	Dodine
Water	28									
Putrescine	37				34	31	30	38	40	46
Spermidine	41				32	31	30	43	38	46
Spermine	43				32	31	30	44	40	47
Arginine	31	34	34	35						
Agmatine	33	32	33	34						
Creatine	31	35	31	35						
Creatinine	27	37	41	45						
Guanidino acetic acid	39	38	42	41						
Dodine	47	44	45	42						

Conditions were same as in Table-9.

activated ATPase, their effect was not additive. This indicates that either they may bind to the membrane at the same site giving activation which could not be enhanced by subsequent binding of the other compound or if they bind at different sites the maximum effect produced by the binding of the two is the same as that given by them individually.

Since sulfhydryl groups of membrane proteins appear to play an important role in membrane function and peroxidase is reported to be inhibited by cysteine (Lotti and Galoppini, 1961) it was of interest to study the interaction of polyamines and guanidines with cysteine and sulfhydryl reagents. When the slices were preincubated with iodoacetate and p-chloromercuri-benzoate (p-CMB) they activated peroxidase by 37 and 77% respectively, whereas cysteine inhibited the enzyme by 32% (Table-11). Incubation of slices first with iodoacetate or p-CMB and then with cysteine after washing completely prevented the increase by sulfhydryl reagents and showed inhibition to the same extent as with cysteine alone. Similar results were obtained with spermine. However, incubation of slices with dodine after sulfhydryl reagents gave an additive effect. The inhibitory effect of spermine was not evident if the slices were treated with it first and then with sulfhydryl reagents but showed 22 and 34% activation respectively. Incubation of slices with dodine first followed by sulfhydryl reagents showed more activation compared to sulfhydryl reagents and dodine alone.

TABLE-11 : Interaction between iodoacetate, p-chloromercuri-benzoate (p-CMB), cysteine, spermine and dodine for peroxidase activity in maize scutellum slices

Treatment Ist / 2nd	Peroxidase activity (units/g tissue)					
	Water	Iodo-acetate	p-CMB	Cysteine	Spermine	Dodine
Water	73					
Iodoacetate	100				89	112
p-CMB	129				98	141
Cysteine	50	58	56		21	59
Spermine	17	16	29	13		
Dodine	101	124	139	59		

Scutellum slices from 4 day germinated seedlings were preincubated (first treatment) for 1 hr in iodoacetate (0.5 mM), p-CMB (0.5 mM), cysteine (0.5 mM), spermine (2 mM) and dodine (2 mM) at 37°. After washing in water, the iodoacetate and p-CMB treated slices were incubated in cysteine, spermine and dodine, while the cysteine treated slices were incubated in spermine and dodine. Similarly the spermine and dodine treated slices were incubated with iodo-acetate, p-CMB and cysteine (second treatment) for 1 hr. The slices after the second incubation were washed with water and used for enzyme assay.

Sulfhydryl reagents also activated ATPase (Table-12) and it was not affected by subsequent treatment with spermine or vice versa. However, incubation of slices with sulfhydryl reagents followed by dodine or vice versa showed an additive effect.

Since in slices only membrane bound peroxidase and ATPase were assayed and they were affected by polyamines and guanidines, it was of interest to investigate whether the two enzymes present on the membranes of other subcellular fractions are also affected by these compounds or not. Subcellular fractions were prepared from scutella excised from seedlings germinated for 4 days. The results reported in Table-13 for peroxidase show that the distribution of the enzyme activity in 1,000g, 10,000g, 40,000g, 105,000g and soluble fractions was 11, 25, 30, 1 and 33% respectively. Preincubation of these fractions with polyamines and guanidines showed that none of the compounds had any effect on the enzyme present in the soluble fraction. Polyamines, however, inhibited all the particulate fractions to various degrees, the maximum effect (approx. 70%) being in the 40,000g fraction. GAA and dodine activated the 1,000g, 10,000g and 40,000g fractions but had no effect on the 105,000g fraction.

A similar study on ATPase (Table-14) showed that maximum activating effect of polyamines and guanidines was

TABLE-12 : Interaction between iodoacetate, p-chloromercuri-benzoate (p-CMB), cysteine, spermine and dodine for ATPase activity in maize scutellum slices.

Treatment Ist / 2nd	ATPase activity (units/g tissue)					
	Water	Iodo-acetate	p-CMB	Cysteine	Spermine	Dodine
Water	27					
Iodoacetate	36				39	53
p-CMB	39				38	52
Cysteine	38	37	39		39	38
Spermine	38	38	38	38		
Dodine	38	54	54	38		

Conditions were same as given in Table-11.

TABLE-13 : Effect of polyamines and guanidines on peroxidase activity in subcellular fractions of maize scutellum

Compound	Peroxidase activity (units/g tissue) in fractions				
	1,000g	10,000g	40,000g	105,000g	Soluble
--	33	76	90	4	102
Putrescine	25	46	40	3	102
Spermidine	22	41	35	3	102
Spermine	20	40	27	3	102
Arginine	33	82	91	4	102
Agmatine	33	76	92	4	102
Creatine	34	77	90	4	100
Creatinine	34	78	89	4	103
Guanidino acetic acid	40	90	100	4	102
Dodine	41	115	114	4	104

Subcellular fractions from scutella of 4 day germinated seedlings were prepared. The fractions were incubated with polyamines and guanidines (1 mM) for 1 hr at 37° before the addition of the other assay components for the enzyme activity test. The homogenate before fractionation had 306 units/g tissue.

TABLE-14 : Effect of polyamines and guanidines on ATPase activity in subcellular fractions of maize scutellum

Compound	ATPase activity (units/g tissue) in fractions				
	1,000g	10,000g	40,000g	105,000g	Soluble
--	35	89	103	46	206
Putrescine	37	120	138	50	204
Spermidine	38	132	145	56	204
Spermine	43	145	157	59	211
Arginine	36	94	108	43	206
Agmatine	35	94	108	43	206
Creatine	35	89	102	44	204
Creatinine	35	89	108	45	206
Guanidino acetic acid	35	115	137	50	204
Dodine	36	150	157	59	211

Conditions were same as given in Table-13.

Homogenate before fractionation had 473 units/g tissue.

for 10,000g and 40,000g fractions. The soluble enzyme which constituted approximately 40% of the total activity in the homogenate was not affected by any of the compounds.

The studies reported till now indicate that polyamines and guanidines modulate the peroxidase and ATPase when they are bound to the membrane. The next approach was to find the nature of binding of these compounds. To investigate this an attempt was made to separate the enzymes from the membrane association. Peroxidase has been reported to be bound ionically as well as covalently on the membrane and it is reported that the ionically bound enzyme can be solubilized by $CaCl_2$ (Haard, 1973). Therefore, $CaCl_2$ and the two surface active agents triton x-100 and sodium deoxycholate were used for solubilization of the two enzymes from mitochondrial (10,000g) and plasma membrane (40,000g) fractions. After treatment with the solubilizing agents the fractions were centrifuged to separate the solubilized and residual fractions which were then incubated with spermine or dodine and assayed for enzyme activity. The results reported in Table-15 for peroxidase show that Ca^{++} could solubilize 80% of the enzyme from both 10,000g and 40,000g fractions, whereas triton x-100 and deoxycholate did not solubilize it. The enzyme which remained in the residual fraction was inhibited by spermine to about 50 and 65% in the 10,000g and 40,000g fractions respectively.

TABLE-15 : Effect of spermine and dodine on peroxidase activity in the 10,000g and 40,000g fractions treated with calcium chloride, triton x-100 and sodium deoxycholate

Fraction	Treatment	Peroxidase activity (units/g tissue)					
		Residue			Supernatant		
		Control	+Spermine	+Dodine	Control	+Spermine	+Dodine
10,000g	--	73	39	105	4	4	5
	$CaCl_2$	13	9	17	60	57	58
	Triton x-100	73	38	108	4	4	4
	Deoxycholate	67	36	99	9	9	9
40,000g	--	86	30	113	4	5	5
	$CaCl_2$	12	4	14	78	77	81
	Triton x-100	85	29	109	4	4	5
	Deoxycholate	78	27	101	9	8	9

The 10,000g and 40,000g fractions from scutella of 4 day germinated seedlings were treated with calcium chloride (0.8 M), triton x-100 and sodium deoxycholate (1%) in the grinding medium for 1 hr at 4° and then centrifuged at 10,000g and 40,000g respectively to separate supernatant and residue fractions. These fractions were then incubated for 1 hr at 37° with spermine or dodine (1 mM) before the addition of other assay components for the enzyme activity test.

The activation of the residual enzyme by dodine was about 45 and 30% in the 10,000g and 40,000g fractions respectively. Spermine and dodine, however, had no effect on the enzyme solubilized by Ca^{++}.

Contrary to the solubilization of peroxidase by Ca^{++}, the ATPase (Table-16) was not solubilized by Ca^{++}. Triton x-100 and deoxycholate, however, could solubilize about 40 and 60% of ATPase activity from the 10,000g and 40,000g fractions respectively. The residual enzyme was still activated by spermine and dodine but the enzyme solubilized by triton x-100 and deoxycholate was not affected by either spermine or dodine. These results indicate that polyamines and guanidines modulate peroxidase and ATPase activity only when they are bound to the membranes and the separation of enzymes from membrane results in the abolition of modulating response.

Attempts were then made to reconstitute the solubilized enzyme from the membrane fraction to ascertain if the reconstituted enzyme will again be modulated by the compounds. These studies could only be carried out with ionically bound peroxidase which was easily solubilized by $CaCl_2$ to the extent of 80% and Ca^{++} could be easily removed by exhaustive dialysis. Attempts made on the reconstitution of ATPase solubilized by triton x-100 or deoxycholate were not

TABLE-16 : Effect of spermine and dodine on ATPase activity in the 10,000g and 40,000g fractions treated with calcium chloride, triton x-100 and sodium deoxycholate

Fraction	Treatment	ATPase activity (units/g tissue)					
		Residue			Supernatant		
		Control	+ Spermine	+ Dodine	Control	+Spermine	+Dodine
10,000g	--	84	109	107	1	1	1
	$CaCl_2$	82	107	106	2	2	2
	Triton x-100	49	66	63	35	35	34
	Deoxycholate	33	43	40	51	51	51
40,000g	--	103	134	130	1	1	1
	$CaCl_2$	101	130	129	1	1	1
	Triton x-100	61	78	76	41	41	41
	Deoxycholate	41	55	52	61	61	61

Conditions were same as given in Table-15.

successful probably because of the difficulty in removing them from the solubilized enzyme fraction. The data reported in Table-17 (Expt.I) show that when peroxidase from plasma membrane fraction having 86 units was solubilized with Ca^{++}, the solubilized fraction after dialysis (Fraction 3) had 67 units, while the residual fraction (2b) had 12 units. When these fractions were mixed, the mixed reconstituted fraction showed 74 units (Fraction 4). Of these 30 units were present in the supernatant obtained after centrifugation and the residue now had 44 units compared to 12 units present earlier (Fraction 2b). This shows that about 50% of the solubilized enzyme could be reattached to the membrane from which it was removed. The reconstituted membrane fraction (Fraction 5b) now again responded to spermine and dodine. In another experiment (Expt.II) reconstitution of the enzyme was attempted not with the enzyme solubilized from the membrane itself but with the cytosolic enzyme and the results showed that the cytosolic enzyme could also attach to the membrane from which the ionically bound peroxidase has been removed. Also, once the cytosolic enzyme gets attached to the membrane it was affected by spermine and dodine in the same manner as ionically bound enzyme.

TABLE-17 : Reconstitution of peroxidase from plasma membrane of maize scutellum and the effect of spermine and dodine on the reconstituted enzyme

Fraction	Peroxidase activity (units) Expt. I*	Expt. II*
1) Plasma membrane	86	86
2) Fraction after solubilization with 0.8 M $CaCl_2$		
(a) Supernatant	73	73
(b) Residue	12	12
3) Fraction 2(a) after dialysis	67	67
4) Reconstitution of fraction 3 with fraction 2(b)/or cytosolic enzyme.	74	72
5) Fraction 4 after centrifugation at 105,000g		
(a) Supernatant	30	32
(b) Residue	44	40
6) Fraction 5(b) + Spermine	18	20
" + Dodine	66	64

* Expt.I : Plasma membrane fraction was treated with 0.8 M $CaCl_2$ for 1 hr and then centrifuged at 105,000g to separate the solubilized enzyme in the supernatant (2a) and the bound enzyme in the

Contd.

TABLE-17 (CONTD.)

residue fraction (2b). The solubilized enzyme (2a) was dialysed thoroughly to remove the Ca ions. The dialysed fraction (3) was then mixed with the residue fraction (2b) in the dialysis bag and was further dialysed for 48 hr. After dialysis the fraction-4 was centrifuged at 105,000g to separate the supernatant (5a) and the residue (5b) fractions. A portion of fraction(5b) was kept for assay and the remaining fraction was pretreated with spermine or dodine (1 mM) for 1 hr before carrying out the enzyme assay.

* Expt.II was same as Expt.I except that the reconstitution at stage 4 was not carried out with fraction-3 i.e. the enzyme solubilized by $CaCl_2$ treatment but with cytosolic enzyme obtained after centrifugation of the homogenate at 105,000g.

TABLE-18 (Contd.)

Plasma membrane peroxidase was solubilized by $CaCl_2$ as described in Table-17. The residue fraction 2(b) was then treated with spermine or dodine (1 mM) for 1 hr and centrifuged at 105,000g to remove the free spermine and dodine from the residue. This residue was then reconstituted with the solubilized enzyme after dialysis (Fraction 3) as described in Table-17. After reconstitution, fraction 4 was centrifuged at 105,000g to separate the supernatant and residue.

Fig 3 : Double reciprocal plot of peroxidase activity at various concentrations of H_2O_2 in the absence and presence of spermine. The mM concentration of spermine is indicated on the lines.

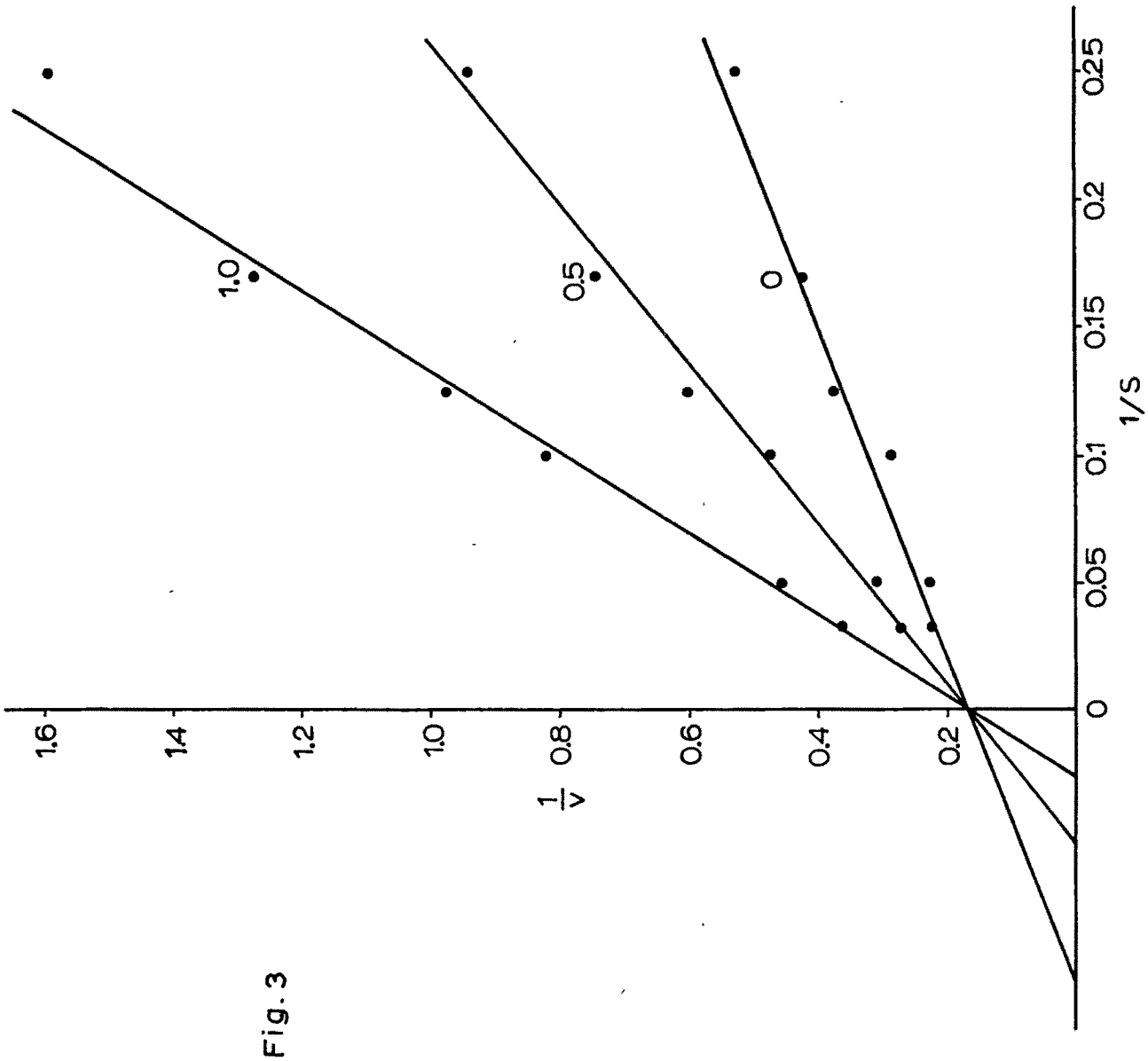


Fig. 3

Fig 4 : Double reciprocal plot of peroxidase activity at various concentrations of H_2O_2 in the absence and presence of dodine. The mM concentration of dodine is indicated on the lines.

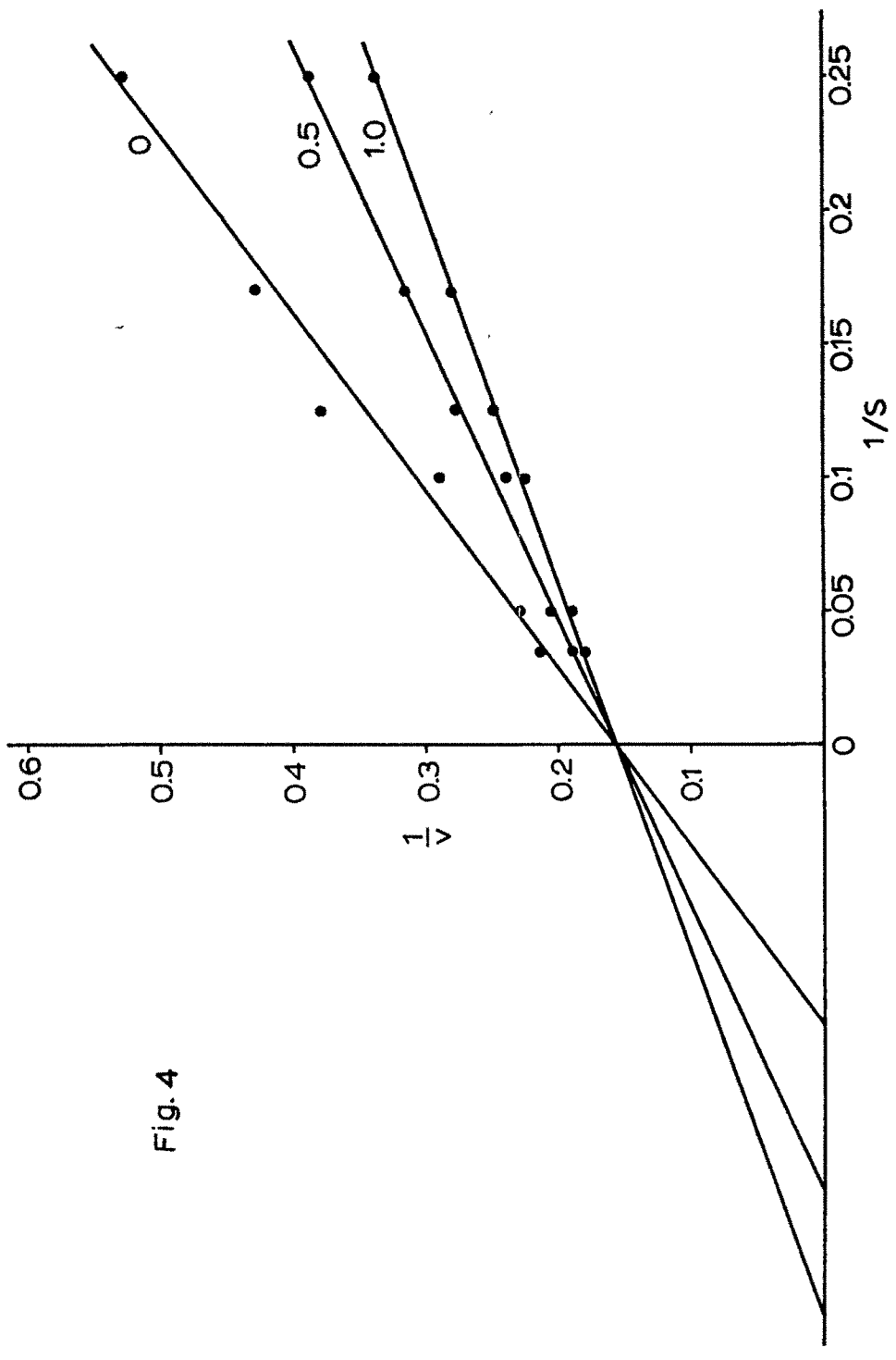


Fig. 4

Fig 5 : Double reciprocal plot of ATPase activity at various concentrations of ATP in the absence and presence of spermine. The mM concentration of spermine is indicated on the lines.

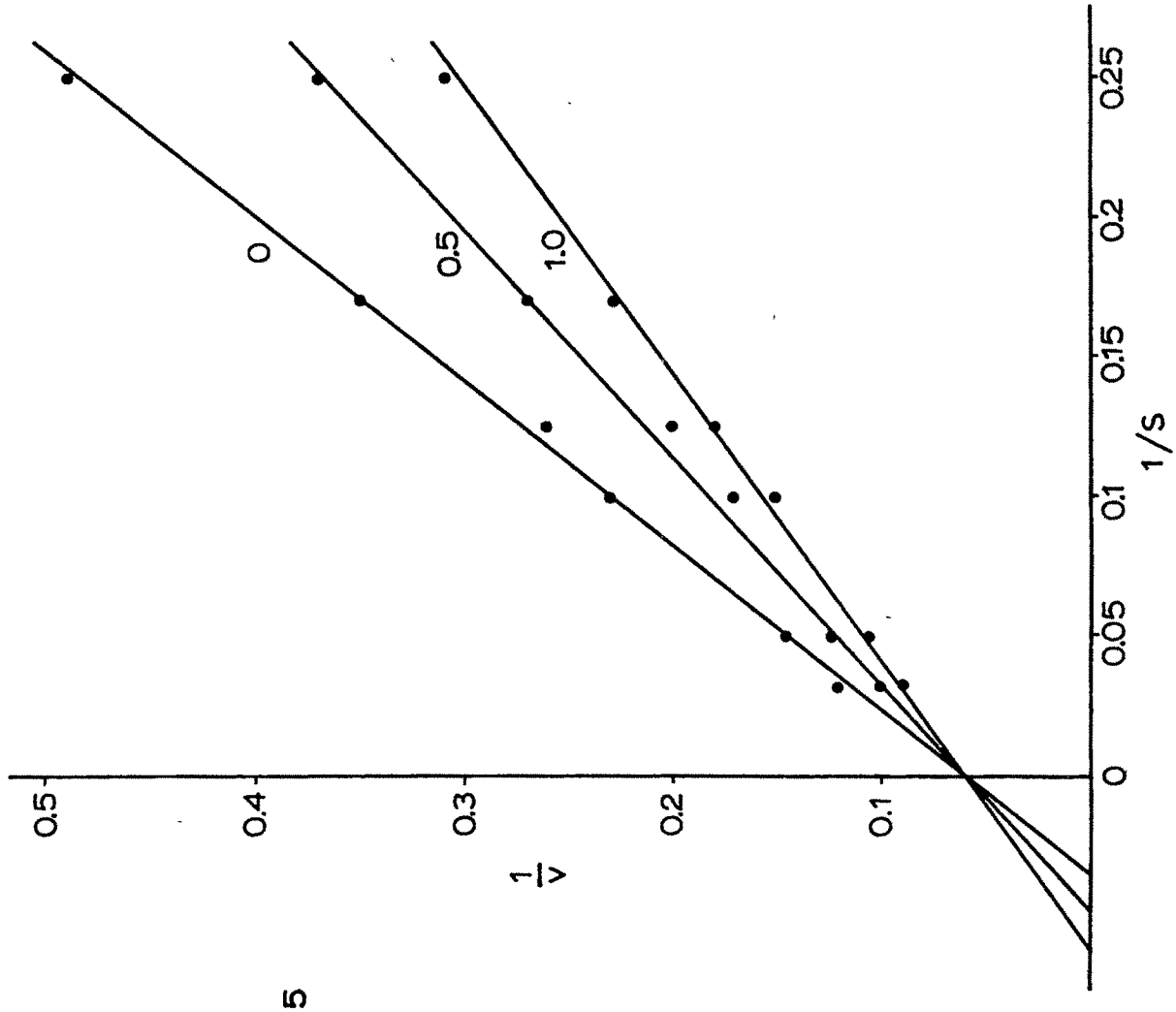
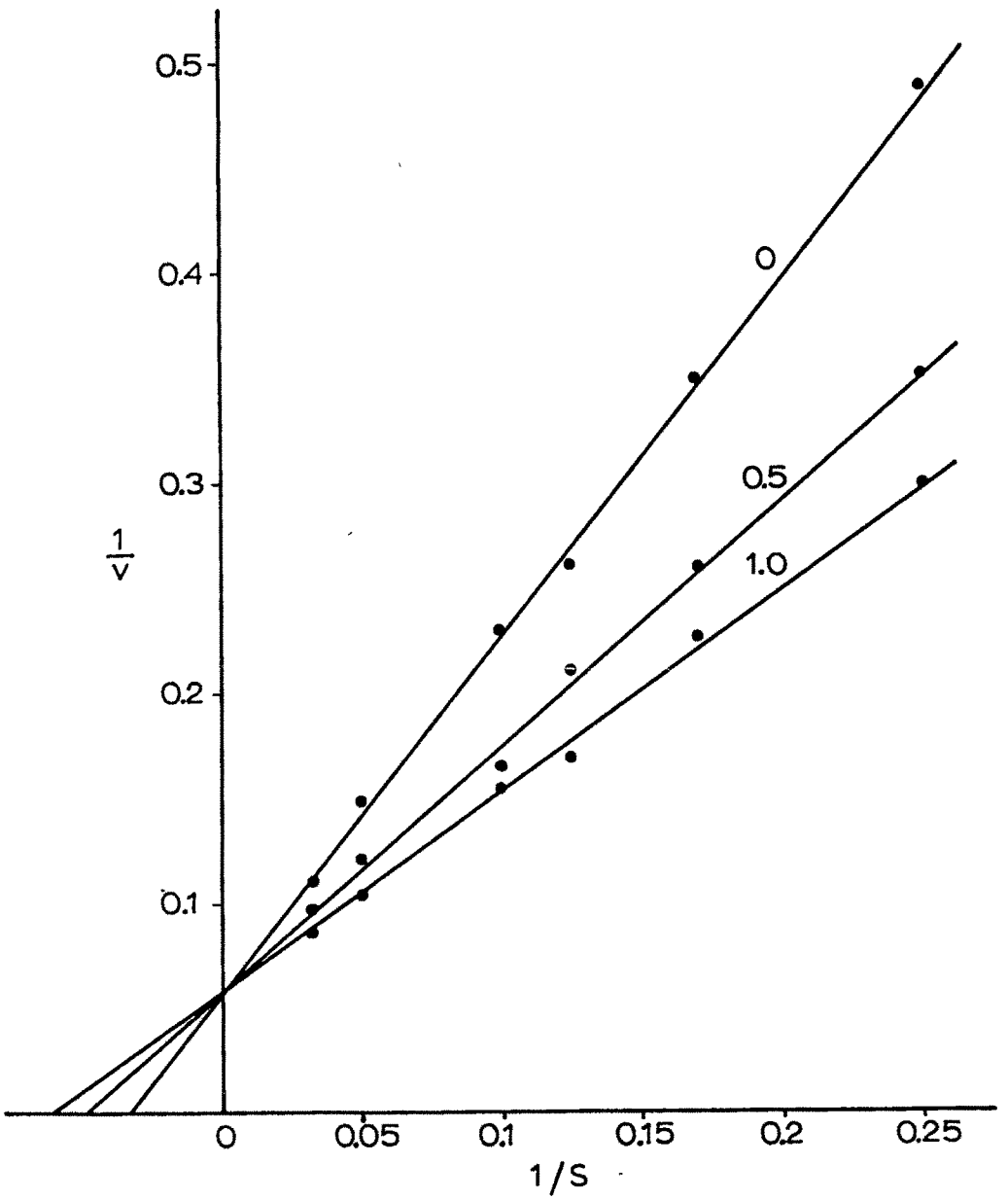


Fig. 5

Fig 6 : Double reciprocal plot of ATPase activity at various concentrations of ATP in the absence and presence of dodine. The mM concentration of dodine is indicated on the lines.

Fig. 6



Effect of polyamines and guanidines on other tissues

In order to investigate whether the effect of polyamines and guanidines on peroxidase and ATPase is specific to maize scutellum only or is a general phenomenon, subcellular fractions from maize root and shoot, barley root, pea and bengalgram embryo were prepared and treated with the compounds as in the case of maize scutellum (Table-13) and assayed for enzyme activity. The results reported in Table-19 and Table-20 show that the peroxidase and ATPase activity from the 40,000g fraction of all the tissues was affected by polyamines and guanidines in a manner almost similar to that of maize scutellum. This study was carried out with other particulate fractions also and they too showed a response similar to that of maize scutellum (data not given). The cytosolic enzymes of none of these tissues were affected by polyamines and guanidines.

TABLE-19 : Effect of polyamines and guanidines on peroxidase activity in 40,000g fraction of different tissues

Compound	Peroxidase activity (units/g tissue) in				
	maize		barley root	pea embryo	bengalgram embryo
	shoot	root			
--	16	21	24	70	86
Putrescine	7	10	10	32	39
Spermidine	6	9	9	24	32
Spermine	5	7	7	20	29
Arginine	17	22	25	77	93
Agmatine	17	22	26	75	87
Creatine	16	21	24	73	90
Creatinine	16	21	25	72	85
Guanidino acetic acid	20	26	32	82	106
Dodine	23	31	37	101	127

TABLE-20 : Effect of polyamines and guanidines on ATPase activity in 40,000g fraction of different tissues

	ATPase activity (units/g tissue) in				
	maize		barley root	pea embryo	bengal-gram embryo
	shoot	root			
--	38	26	46	150	56
Putrescine	50	34	63	201	71
Spermidine	53	36	67	215	74
Spermine	57	39	72	231	81
Arginine	41	26	51	174	61
Agmatine	40	27	49	159	58
Creatine	38	26	47	159	56
Creatinine	39	27	48	154	55
Guanidino acetic acid	49	33	57	202	71
Dodine	58	39	65	237	83

Phytochrome mediation and its interaction with polyamines and guanidines in regulating peroxidase and ATPase activity in maize scutellum

Studies reported in the previous section have shown that polyamines and guanidines can influence the activity of the membrane bound peroxidase and ATPase. Phytochrome, which is involved in controlling several biochemical processes and enzymes including peroxidase and ATPase, is also known to be bound to the membranes. It was of interest, therefore, to investigate the interaction, if any, between polyamines and guanidines with phytochrome in modulating these enzymes.

To demonstrate the phytochrome mediation of peroxidase and ATPase, the scutella excised along with the embryo from the seedlings germinated in dark for different days were exposed to red (R) far-red (FR) or red followed by far-red (R-FR)lights. After exposure the scutella were incubated in dark. At specified periods the embryo was removed and slices from the scutella were prepared and used for the assay of peroxidase and ATPase activity. In all the experiments the time of exposure to light was taken as 0 hr.

The results reported in Table-21 for peroxidase show that red or far-red light had no effect on the development of peroxidase in the scutella excised from 1 day germinated seedlings. Scutella excised from 2 day germinated seedlings showed an increase in peroxidase activity in red light treated group starting from 8 hr. The maximum increase in activity was to the extent of about 20%. The red light response was maximum in the scutella from 3 day germinated seedlings where it increased peroxidase activity by about 40% from 4 hr onwards and it decreased in the scutella from the seedlings germinated for 4 days. The red light effect persisted throughout the period of incubation (36 hr) though it was considerably decreased at later periods in the scutella from 4 day germinated seedlings. Far-red light alone did not have any effect on the enzyme but if the red light was followed by an immediate far-red light treatment, the increase in peroxidase activity brought about by red light was completely abolished, confirming the phytochrome mediation of the enzyme.

ATPase activity (Table-22) also showed an activation by red light and the pattern was almost similar to peroxidase except that the effect did not persist till 36 hr of incubation but started decreasing after 12 hr.

TABLE-21: Effect of red and far-red light on the development of peroxidase activity in maize scutellum

Scutella removed from seeds germinated in dark for (days)	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
1	D	5	7	10	17	34	50
	R	5	7	10	18	34	50
	FR	5	7	10	19	34	50
	R-FR	5	7	10	19	34	51
2	D	36	42	51	92	101	114
	R	36	45	57	106	120	123
	FR	36	42	53	93	103	114
	R-FR	36	43	54	95	105	117
3	D	65	75	81	107	114	116
	R	92	103	114	141	148	154
	FR	67	75	82	108	114	118
	R-FR	68	76	84	109	114	121
4	D	75	83	92	113	118	127
	R	92	100	110	129	132	136
	FR	77	83	93	114	119	129
	R-FR	81	87	98	118	122	130

Contd.

TABLE-21 (Contd.)

Scutella from seeds germinated for different days in dark were excised and kept in Petridishes containing moist filter papers either in dark (D) which served as control or exposed to red light (R) for 5 min, far-red light (FR) for 15 min or red followed immediately by far-red light (R-FR). After exposure to lights the scutella were transferred to dark and allowed to incubate at 22°. At specified periods after exposure slices were prepared, washed in water and used for enzyme assay.

TABLE-22 : Effect of red and far-red light on the development of ATPase activity in maize scutellum

Scutella removed from seeds germinated in dark for (days)	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
1	D	10	13	16	27	28	29
	R	11	13	15	27	28	29
	FR	11	13	16	27	28	29
	R-FR	10	13	16	27	28	29
2	D	14	17	20	34	35	36
	R	15	19	22	40	40	41
	FR	14	17	19	35	34	37
	R-FR	14	17	20	35	36	37
3	D	17	21	23	40	43	45
	R	23	28	31	47	48	50
	FR	18	21	24	41	43	45
	R-FR	18	22	25	41	43	46
4	D	30	34	38	52	54	57
	R	34	38	41	55	57	59
	FR	31	35	39	54	57	58
	R-FR	31	34	39	54	55	57

Conditions were same as in Table-21.

These studies demonstrated that the response of scutellum tissue to phytochrome initially increased with period of germination upto 3 days but again decreased with further germination. In fact the scutella from seeds germinated for 5 day onwards did not show any red light mediated response (data not given). Scutella from seedlings germinated for 3 days were, therefore, used for studying the effect of polyamines and guanidines on phytochrome mediation. For these studies the scutella along with the embryo were either transferred to Petridishes containing compounds and exposed to lights immediately or they were first exposed to light, transferred to dark and then 2 hr after exposure they were transferred to Petridishes containing compounds. In another set the scutella were first treated with the compounds in dark and after 2 hr they were exposed to light and again kept in dark for incubation. Thus the experimental set up was such that either the exposure to light and compounds was done simultaneously or the light was given first followed 2 hr later by compounds or the compounds were given first and the lights were given after 2 hr.

The results reported in Table-23 for peroxidase, where light exposure was given immediately after transferring the scutella to Petridishes containing compounds, show that putrescine, spermidine and spermine though had no effect

TABLE-23 : Effect of polyamines and guanidines on phytochrome mediated response of peroxidase activity in maize scutellum

Compound	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
--	D	66	71	77	99	105	113
	R	90	102	110	147	157	162
	FR	70	78	79	99	108	113
	R-FR	70	78	80	100	109	115
Putrescine	D	67	74	75	97	103	108
	R	90	100	105	121	125	128
	FR	71	79	81	101	105	113
	R-FR	70	78	81	101	106	116
Spermidine	D	65	73	76	95	108	110
	R	88	99	103	117	124	125
	FR	67	75	78	100	112	115
	R-FR	66	76	81	101	110	111
Spermine	D	65	72	75	96	108	110
	R	89	95	103	118	121	130
	FR	69	79	79	101	116	113
	R-FR	67	78	80	98	113	115

Contd.

TABLE-23 (Contd.)

Compound	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
Guanidino acetic acid	D	67	79	93	138	156	166
	R	91	101	105	147	157	167
	FR	67	76	83	100	109	114
	R-FR	66	77	84	101	112	115
Dodine	D	66	80	93	139	156	167
	R	91	99	105	148	158	168
	FR	68	75	83	101	110	113
	R-FR	69	76	84	102	112	115

Scutella along with the embryo from 3 day old dark grown seedlings were removed and were placed in Petridishes containing 2 layers of filter papers moistened with compounds (2 mM). The scutella were then either kept in dark (D) to serve as control or immediately exposed to red light (R) for 5 min, far-red light (FR) for 15 min or red followed by far-red light (R-FR). After exposure to light the scutella were transferred to dark and allowed to incubate at 22°. At specified intervals after exposure slices were prepared, washed in water and used for enzyme assay.

on the dark control group but partially decreased the red light induced increase in peroxidase activity from 12 hr onwards. The far-red light completely reversed the red light response to the dark control level. GAA and dodine, however, increased peroxidase activity even in the dark control group. The red light had no additive effect in presence of these two compounds. Also far-red light either alone or after red light was again able to reverse the increase brought about by the guanidino compounds. In the second group, where light exposure was given prior to treatment with compounds, the results (Table-24) were similar to the earlier group where light and compounds were given simultaneously. In the third group, where scutella were treated with the compounds 2 hr before exposure to lights showed (Table-25) that the general pattern of the response was same as in the previous groups, except that the effect was evident from 4 hr onwards and the polyamines could completely reverse the red light induced increase in peroxidase activity.

ATPase activity studied under the similar set up showed that when the light exposure and treatment with compounds was carried out simultaneously (Table-26) or when the light exposure was given 2 hr before treatment with compounds (Table-27) or when the compounds were given 2 hr before light exposure (Table-28) the red light induced ATPase activity was not affected by polyamines and the

TABLE-24 : Effect of polyamines and guanidines on phytochrome mediated response of peroxidase activity in maize scutellum

Compound	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
--	D	63	72	76	101	107	112
	R	91	102	110	148	160	167
	FR	68	78	84	111	118	118
	R-FR	70	79	86	115	120	121
Putrescine	D	61	75	78	98	110	118
	R	88	105	108	123	125	129
	FR	68	78	83	108	115	122
	R-FR	70	79	84	113	117	123
Spermidine	D	62	76	80	101	108	115
	R	90	105	109	120	125	128
	FR	68	79	81	108	110	117
	R-FR	68	78	79	106	112	118
Spermine	D	64	73	76	99	105	112
	R	91	105	108	122	125	129
	FR	68	79	79	105	110	115
	R-FR	67	78	81	106	112	116

Contd.

TABLE-24 (Contd.)

Compound	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
Guanidino acetic acid	D	63	78	91	137	151	163
	R	88	101	109	154	160	166
	FR	68	76	79	105	110	115
	R-FR	67	75	84	105	112	118
Dodine	D	65	79	93	140	153	167
	R	87	99	109	151	159	166
	FR	67	75	81	106	112	115
	R-FR	68	77	83	106	112	118

Scutella along with the embryo from 3 day old dark grown seedlings were removed. They were either kept in dark (D) to serve as control or exposed to red light (R) for 5 min, far-red light (FR) for 15 min or red followed by far-red light (R-FR). Two hr after exposures to light, the scutella were transferred to Petridishes containing 2 layers of filter papers moistened with 2 mM compounds and allowed to incubate at 22° in dark. At specified intervals after exposure slices were prepared, washed in water and used for enzyme assay.

TABLE-25 : Effect of polyamines and guanidines on phytochrome mediated response of peroxidase activity in maize scutellum

Compound	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
--	D	64	73	78	106	109	114
	R	92	104	114	148	148	151
	FR	68	78	87	117	121	128
	R-FR	68	78	86	120	124	129
Putrescine	D	67	75	76	109	113	114
	R	71	74	79	110	115	117
	FR	67	76	76	112	118	121
	R-FR	68	76	77	113	116	122
Spermidine	D	66	73	73	105	110	114
	R	69	72	74	104	111	116
	FR	68	75	71	105	109	116
	R-FR	69	74	72	105	110	115
Spermine	D	67	74	78	106	112	117
	R	66	73	79	107	111	116
	FR	66	73	78	107	109	115
	R-FR	67	74	79	106	110	115

Contd.

TABLE-25 (Contd.)

Compounds	Light treatment	Peroxidase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
Guanidino acetic acid	D	86	103	115	149	156	166
	R	88	104	115	149	157	170
	FR	66	75	83	112	115	120
	R-FR	68	76	84	112	116	122
Dodine	D	87	101	114	149	162	167
	R	87	105	115	149	162	168
	FR	67	76	84	112	116	120
	R-FR	69	77	85	113	117	123

Scutella along with the embryo from 3 day old dark grown seedlings were removed. They were placed in Petridishes containing 2 layers of filter paper moistened with 2 mM compounds. Two hr after placing the scutella in the Petridishes, they were exposed to red light (R) for 5 min far-red light (FR) for 15 min or red followed by far-red light (R-FR) and allowed to incubate at 22° in dark. At specified intervals after exposure, slices were prepared, washed in water and enzyme activity was assayed.

TABLE-26 : Effect of polyamines and guanidines on phytochrome mediated response of ATPase activity in maize scutellum

Compound	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
--	D	16	20	23	39	42	45
	R	19	25	29	47	48	49
	FR	17	21	24	40	44	47
	R-FR	17	20	24	41	44	47
Putrescine	D	17	20	22	40	43	46
	R	20	26	28	46	49	51
	FR	17	22	25	42	45	47
	R-FR	18	21	25	41	44	48
Spermidine	D	15	19	22	39	42	45
	R	19	24	29	46	49	51
	FR	17	21	24	41	44	47
	R-FR	17	21	25	42	44	47
Spermine	D	17	20	22	40	43	47
	R	20	25	31	39	51	51
	FR	18	21	26	43	45	47
	R-FR	18	21	27	44	44	48

Contd.

TABLE-26 (Contd.)

Compound	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
Guanidino acetic acid	D	16	22	25	43	48	50
	R	21	25	29	47	49	51
	FR	17	21	26	42	45	47
	R-FR	18	21	25	41	44	47
Dodine	D	17	22	28	44	48	51
	R	20	25	31	46	50	51
	FR	18	21	25	42	43	46
	R-FR	17	21	25	42	43	46

Conditions were same as in Table-23.

TABLE-27 : Effect of polyamines and guanidines on phytochrome mediated response of ATPase activity in maize scutellum

Compound	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
--	D	17	21	23	39	43	46
	R	21	26	30	48	50	51
	FR	18	22	25	41	45	48
	R-FR	18	23	26	41	45	48
Putrescine	D	16	22	25	40	42	47
	R	21	25	31	48	49	51
	FR	18	23	25	41	44	48
	R-FR	18	23	24	41	44	47
Spermidine	D	17	23	25	40	42	47
	R	22	25	31	48	50	52
	FR	18	23	25	41	43	49
	R-FR	18	22	24	40	43	49
Spermine	D	15	19	22	39	42	45
	R	20	25	29	46	49	51
	FR	18	22	24	41	45	47
	R-FR	17	21	25	41	44	47

Contd.

TABLE-27 (Contd.)

Compound	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
Guanidino acetic acid	D	18	22	25	43	46	49
	R	20	26	29	46	49	51
	FR	18	21	23	41	42	45
	R-FR	18	22	24	40	43	46
Dodine	D	17	21	23	41	46	49
	R	20	24	28	45	49	51
	FR	17	20	22	40	42	45
	R-FR	18	21	23	40	42	46

Conditions were same as Table-24.

TABLE-28 : Effect of polyamines and guanidines on phytochrome mediated response of ATPase activity in maize scutellum

Compound	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
--	D	17	20	22	40	43	46
	R	19	25	29	47	48	49
	FR	18	22	25	41	45	48
	R-FR	17	21	25	42	44	47
Putrescine	D	16	21	23	39	43	46
	R	21	25	31	48	49	51
	FR	18	22	24	41	45	47
	R-FR	18	22	24	40	43	49
Spermidine	D	17	23	25	40	42	47
	R	20	25	31	49	51	51
	FR	17	21	24	41	44	47
	R-FR	18	21	27	44	44	48
Spermine	D	16	20	23	39	42	45
	R	20	26	28	46	49	51
	FR	17	21	24	40	44	46
	R-FR	17	21	25	42	44	47

Contd.

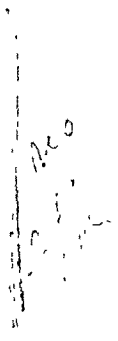
TABLE-28 (Contd.)

Compound	Light treatment	ATPase activity (units/g tissue) after exposure to light (hr)					
		4	8	12	24	30	36
Guanidino acetic acid	D	17	22	29	45	49	51
	R	20	23	30	46	49	51
	FR	17	20	23	42	43	45
	R-FR	17	21	25	41	43	45
Dodine	D	17	23	29	45	48	51
	R	21	25	30	46	49	51
	FR	17	21	25	42	42	45
	R-FR	18	22	25	43	44	45

Conditions were same as Table-25.

increase by guanidines in the dark control group was of the same order as with red light treated group. The activation by the red light or by the guanidino compounds was reversed by far-red light.

Studies reported in the earlier sections have shown that polyamines inhibit peroxidase and activate ATPase activity while the guanidino compounds activated both the enzymes when the slices were preincubated with them. However, in the studies with phytochrome mediation where whole scutella along with the embryo were treated with these compounds before assaying the activity in slices showed that polyamines have no effect in the dark control group, though guanidino compounds still showed some activation.



To investigate whether the negative response of polyamines in the whole scutella along with the embryo could be due to their break down in scutellum tissue during the long period of incubation, the scutella were assayed for the polyamines oxidase activity. The results showed that scutellum tissue has no polyamine oxidase activity but the embryo has polyamine oxidase activity. Thus it appears that the negative response of polyamines in the whole scutellum may not be due to breakdown of polyamines but could be either due to a slow intake or its transport to the embryo where it may be broken down. To investigate this further, in an experiment the scutella with embryo or without embryo were incubated

with the compounds and light exposures were given. The results reported in Table-29 show that in case where the embryo was present with the scutellum during the treatment, the spermine had no effect on the dark control group but in the group where the embryo was removed before starting the treatment, spermine could decrease the peroxidase and increase the ATPase as expected, though, the percentage effect was less than in the slices. Another interesting observation was that, if the embryo was removed from the scutellum before the light treatment there was no phytochrome response on the two enzymes suggesting that the light perception is passed on to the scutellum tissue through the embryo. Dodine, however, had similar effect in both the groups but far-red light could not reverse the effect in the group where embryo was not present.

Since polyamines and guanidines modulate only the bound peroxidase and ATPase activity and phytochrome appears to interact with these compounds in controlling the activity, it was of interest to see whether only the enzymes bound to the membranes are affected. The scutella were exposed to light and compounds simultaneously and after homogenization, the particulate (105,000g residue) and supernatant fractions were separated and assayed for enzyme activity. The results reported in Table-30 show that red light activated both peroxidase and ATPase in particulate as well as in the soluble fractions and the activation was reversed by the subsequent far-red light treatment. Spermine, however, decreased the red light

TABLE-29 : Effect of spermine and dodine on phytochrome mediation of peroxidase and ATPase activity in maize scutellum with embryo and without embryo

Compound	Light treatment	Enzyme activity (units/g tissue) in scutella incubated							
		with embryo				without embryo			
		Peroxidase		ATPase		Peroxidase		ATPase	
		at hr				at hr			
		24	30	24	30	24	30	24	30
--	D	109	127	33	37	107	127	32	36
	R	151	189	40	46	109	119	33	35
	R-FR	104	119	34	39	104	114	31	37
Spermine	D	101	117	32	35	79	97	40	45
	R	122	132	42	48	74	102	41	45
	R-FR	104	115	34	36	71	93	39	43
Dodine	D	144	184	39	45	142	187	42	46
	R	151	189	41	46	142	189	42	48
	R-FR	107	129	33	38	140	182	40	44

Scutella with embryo and without embryo were taken from 3 day germinated seedlings and were placed in petridishes containing 2 layers of filter papers moistened with 2 mM spermine or dodine. The scutella were then either kept in dark (D) to serve as control or immediately exposed to red light (R) for 5 min, or red followed by far-red (R-FR) for 5 and 15 min, and transferred to dark and allowed to incubate at 22°. At specified intervals after exposure slices were prepared from the scutellum and used for enzyme assay.

TABLE-30 : Effect of spermine and dodine on phytochrome mediated response of peroxidase and ATPase activity in particulate and soluble fractions from maize scutellum

Compound	Light treatment	Enzyme activity (units/g tissue)			
		Peroxidase		ATPase	
		in fractions			
		Particulate	Solube	Particulate	Soluble
--	D	194	111	258	274
	R	248	132	346	361
	FR	196	112	263	279
	R-FR	206	114	274	299
Spermine	D	196	103	258	274
	R	204	132	355	361
	FR	198	113	268	274
	R-FR	202	114	276	315
Dodine	D	250	111	335	263
	R	250	131	351	377
	FR	196	112	276	274
	R-FR	206	113	276	289

Contd.

TABLE-30 (Contd.)

Scutella were removed from 3 day old dark grown seedlings and were placed in Petridishes containing 2 layers of filter paper moistened with 2 mM spermine and dodine. The scutella were then either kept in dark (D) to serve as control or immediately exposed to red light (R) for 5 min, far-red light (FR) for 15 min. or red followed by far-red light (R-FR). After exposure to lights the scutella were transferred to dark and allowed to incubate at 22°. After 24 hr particulate (105,000g residue) and soluble (105,000g supernatant) fractions were prepared and enzymes were assayed.

induced peroxidase activity of the particulate fraction but not that of the soluble fraction. The red light induced ATPase activity was, however, not affected by spermine in the particulate or the soluble fraction. Dodine increased the peroxidase and ATPase activity of the particulate fraction but not of the soluble fraction in the dark control group. This increase was to the same extent as with red light treatment and was reversed by the subsequent far-red light. These results confirm that even though phytochrome can increase the activity of the two enzymes in both particulate and soluble fractions, polyamines and guanidines interact with phytochrome only for the bound enzymes.

Effect of plant hormones on peroxidase and ATPase activity in maize scutellum

Plant hormones are known to affect peroxidase and ATPase activity and some of their physiological effects are mediated through membranes. Studies were carried out to see whether plant hormones could modulate membrane bound peroxidase and ATPase from maize scutellum slices and their possible interaction with polyamines and guanidines. Scutellum slices were incubated with different concentrations of indole acetic acid, gibberellic acid, kinetin and etherel

and after washing the slices were used for enzyme assay. The results reported in Table-31 for peroxidase show that indole acetic acid, gibberellic acid and kinetin had no effect on peroxidase activity but etherel inhibited it. The inhibition increased with increasing concentration. In a similar study on ATPase (Table-32) none of the compounds were found to have any effect.

Since etherel inhibited peroxidase and GAA and dodine were found to activate it, studies were carried out to demonstrate the interaction, if any, between etherel and guanidines. Scutellum slices were either incubated in etherel first and then in GAA or dodine after washing or vice versa. The results reported in Table-33 show that when slices were first incubated in etherel and then in guanidino compounds the inhibitory effect of etherel was partially reversed by guanidino compounds. However, when the slices were first incubated in guanidino compounds and then in etherel, the activating effect of the guanidino compounds was completely reversed by etherel and it inhibited to the same extent as alone.

TABLE-31 : Effect of hormone concentration on peroxidase activity in maize scutellum slices

Concentration (µM)	Peroxidase activity (units/g tissue)			
	Indole acetic acid	Gibberellic acid	Kinetin	Etherel
0	80			
10	70	83	80	63
25	70	79	79	18
50	66	81	80	5
100	65	79	79	4

Scutellum slices from 4 day germinated seedlings were preincubated with the hormones at different concentrations for 1 hr. After incubation the slices were washed in water and used for enzyme assay.

TABLE-32 : Effect of hormone concentration on ATPase activity in maize scutellum slices

Concentration (μM)	ATPase activity (units/g tissue)			
	Indole acetic acid	Gibberellic acid	Kinetin	Etherel
0	30			
10	30	31	26	29
25	29	31	26	28
50	29	30	26	26
100	28	28	26	26

Conditions were same as in Table-31.

TABLE-33 : Interaction between etherel, guanidino acetic acid and dodine for peroxidase activity in maize scutellum

Treatment Ist / 2nd	Peroxidase activity (units/g tissue)			
	Water	Etherel	Guanidino acetic acid	Dodine
Water	84	23	115	119
Etherel	6		9	7
Guanidino acetic acid		75		
Dodine		97		

Scutella from 4 day germinated seedlings were used. Slices were preincubated in etherel (50 µM), guanidino acetic acid (2 mM) and dodine (2 mM) (first treatment) for 1 hr at 37°. After washing in water, the etherel treated slices were incubated in guanidino acetic acid and dodine whereas guanidino acetic acid and dodine treated slices were incubated in etherel (second treatment) for 1 hr at 37°. After the second treatment the slices were washed in water and used for enzyme assay.