RESULTS

RESULTS

Effect of nitrogen sources on the seedling growth and development of enzymes :

The level of nitrogen assimilatory enzymes is known to be dependent upon the nitrogen source supplied. In the present study the effect of nitrate and ammonium as the nitrogen sources was studied on the growth of the seedling as well as on the level of the enzymes.

Seeds were grown in continuous light or dark conditions in the absence or presence of a nitrogen source. Fresh weight of the embryonal axis (as an index of growth) and the developmental pattern of enzymes was determined during the course of germination.

The fresh weight of the axis (Fig. 1) increased with germination. Nitrate had no effect during the first three days of germination. However, an increase in weight was observed from 4th day in both light and dark grown seeds. With ammonium, however, an inhibition of growth was observed in dark grown seeds from 1st day of germination which persisted throughout the period of germination.

Developmental pattern of the enzymes showed that in the absence of a nitrogen source the NR activity was low

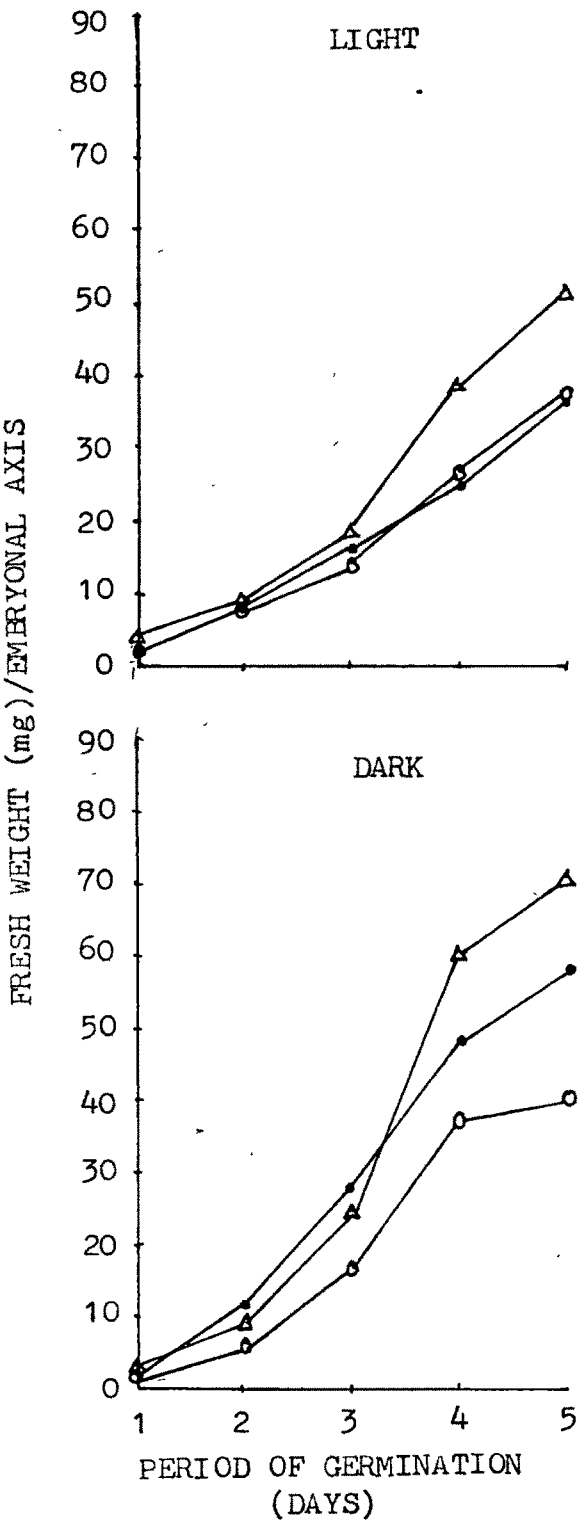

Fig. 1 : Effect of nitrogen sources on the growth of embryonal axis during germination of radish seeds.
Control, •—• ; +KNO_3, △—△ ; +NH_4Cl, o—o .

(Fig. 2) which increased slightly upto 3rd day and then remained almost constant during the period of germination. Light grown seeds had about 2 fold higher activity than the dark grown ones. In the presence of nitrate, the activity increased by almost 5 fold in both light and dark grown seeds on the 2nd day and declined gradually thereafter. The level of the enzyme in light on the 2nd day was almost twice that in dark grown seeds. Ammonium brought about a slight increase in NR activity in light as well as dark grown seeds.

The level of NiR (Fig. 3) was low in the absence of a nitrogen source which increased slightly between 2nd and 3rd day of germination and declined thereafter in both light and dark grown seeds. Nitrate brought about a 2-3 fold increase in both light and dark grown seeds and the enzyme activity in light grown seeds was almost twice that in dark grown seeds. The activity increased till the 3rd day in light grown seeds while it reached a maximum on the 4th day in dark grown ones. Ammonium showed no effect in dark grown seeds but increased the enzyme activity slightly in light grown seeds.

A very low level of GS was observed in the absence of a nitrogen source (Fig. 4) in both light and dark grown seeds which remained almost constant throughout the period

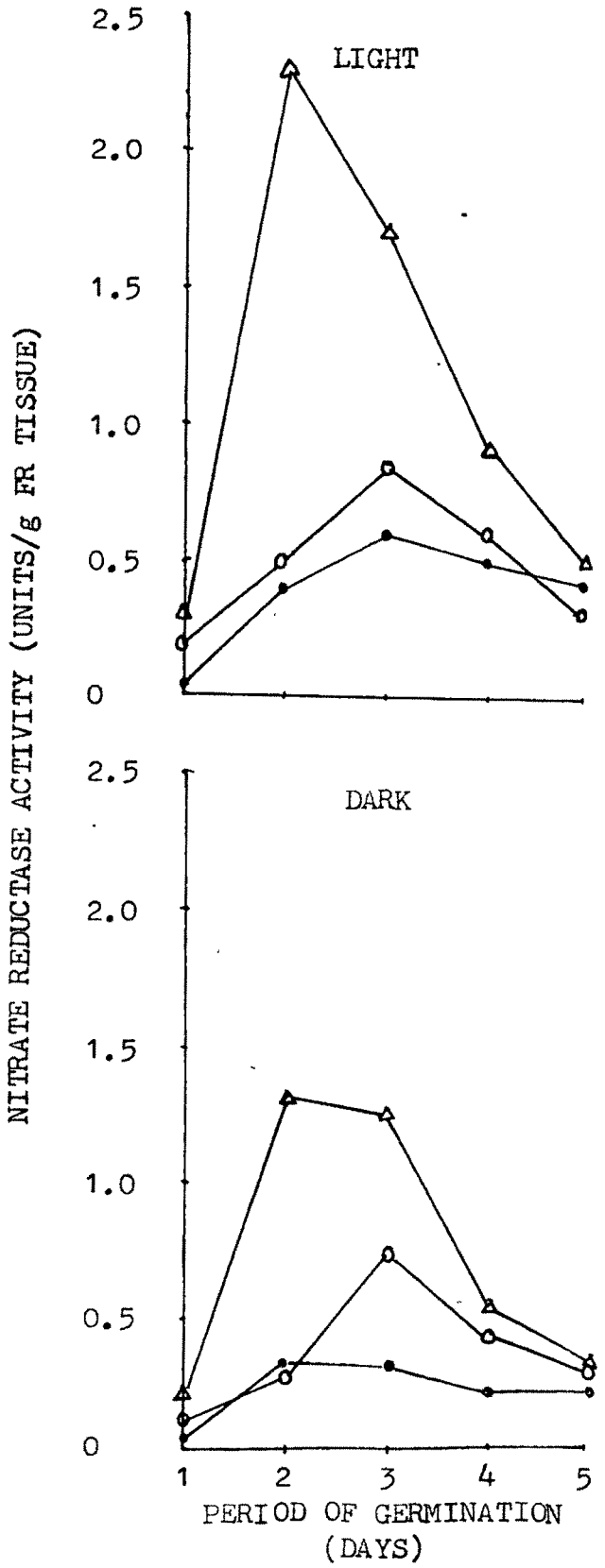

Fig. 2 : Effect of nitrogen sources on nitrate reductase activity during germination of radish seeds. Control, ●—● ; +KNO_3, △—△ ; +NH_4Cl, o—o .

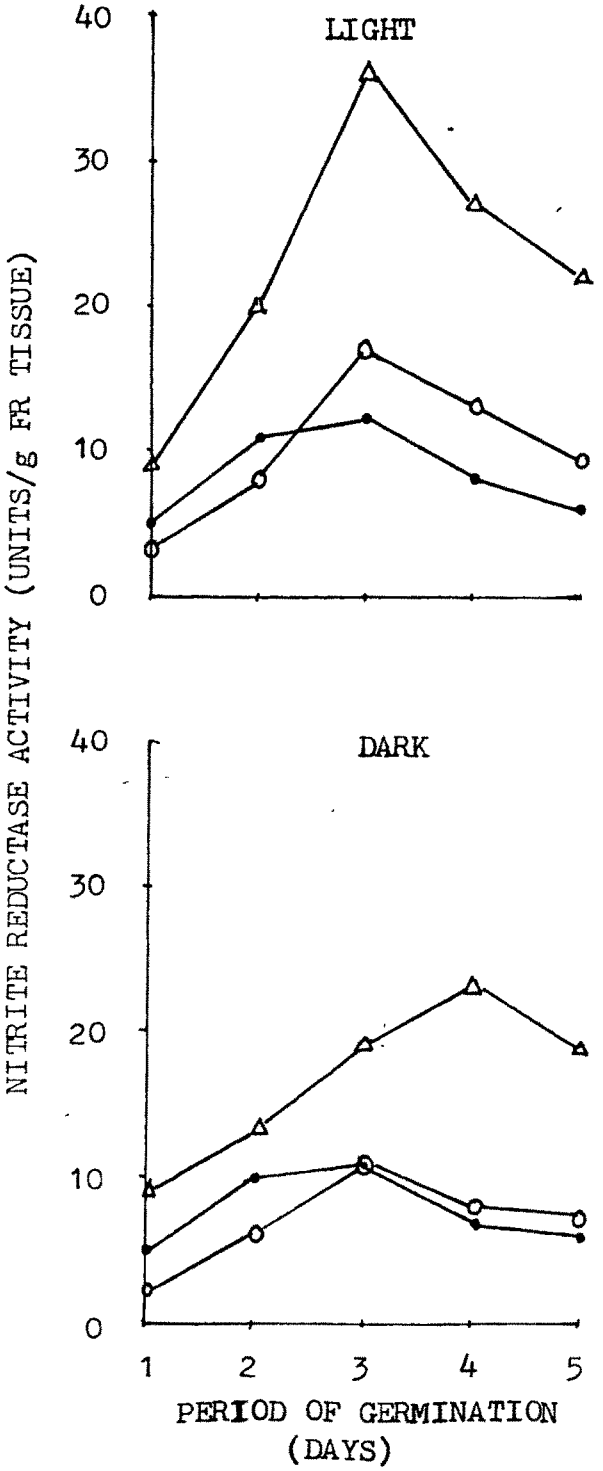

Fig. 3 : Effect of nitrogen sources on nitrite reductase activity during germination of radish seeds. Control, •—• ; +KNO_3, △—△ ; +NH_4Cl, o—o .

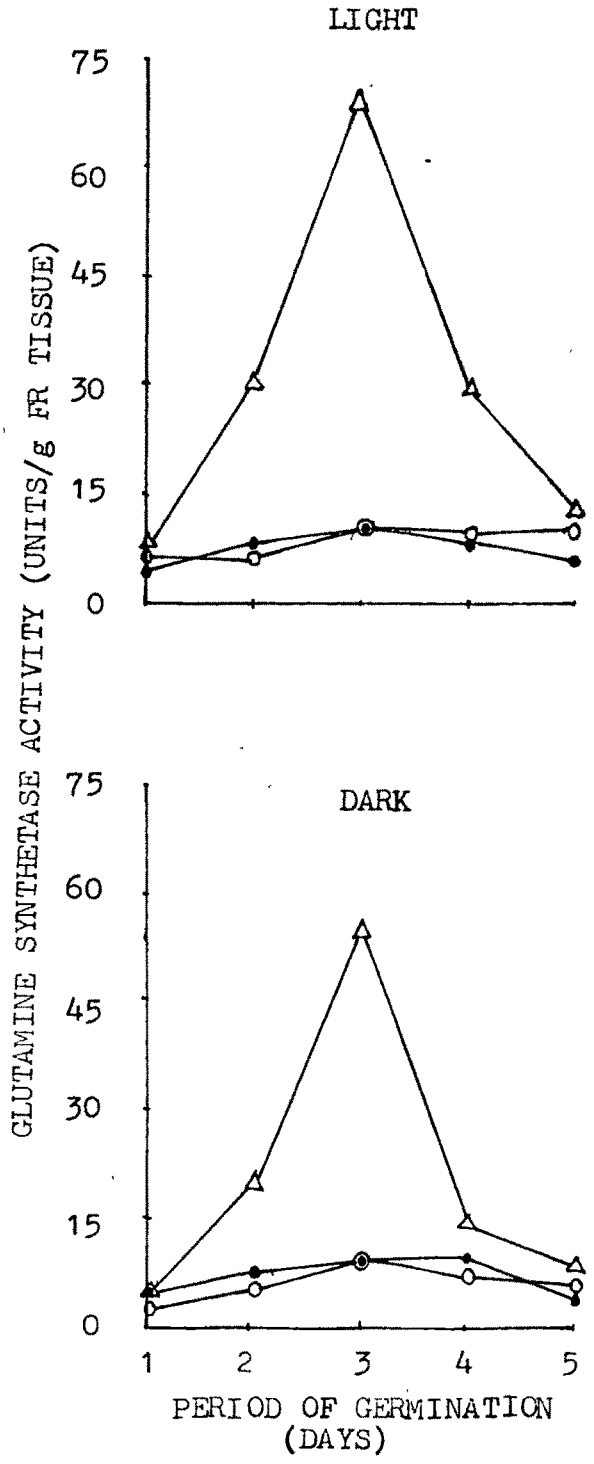

Fig. 4 : Effect of nitrogen sources on glutamine synthetase activity during germination of radish seeds.
Control, •—• ; $+KNO_3$, Δ—Δ ; $+NH_4Cl$, o—o .

of germination. However, it increased by 6-7 fold in the presence of nitrate on the 3rd day in both light and dark grown seeds and declined sharply thereafter. Light grown seeds showed about 1.5 fold higher activity than in dark grown ones. In contrast to nitrate, ammonium had no effect on GS activity in light or dark grown seeds.

A high GDH level was observed on the 1st day of germination even in the absence of a nitrogen source (Fig.5) which declined gradually by 5th day in both light and dark grown seeds. Light did not have any effect on the level of GDH. Addition of nitrate brought about a 1.5-2 fold increase in enzyme activity in light as well as in dark grown seeds. Ammonium was more effective than nitrate in both light and dark. The pattern of change in GDH in presence of nitrate or ammonium over the period of germination, however, was same as in the control group.

A high protease activity was obtained in the control group on the 1st day of germination which increased upto the 2nd and 3rd day in light and dark grown seeds respectively and then gradually declined (Fig. 6). Light did not have any effect on the enzyme level. The supply of nitrate lowered the level of enzyme in dark grown seeds but had very little effect in light grown seeds. Ammonium grown seeds also showed lower activity as compared to the

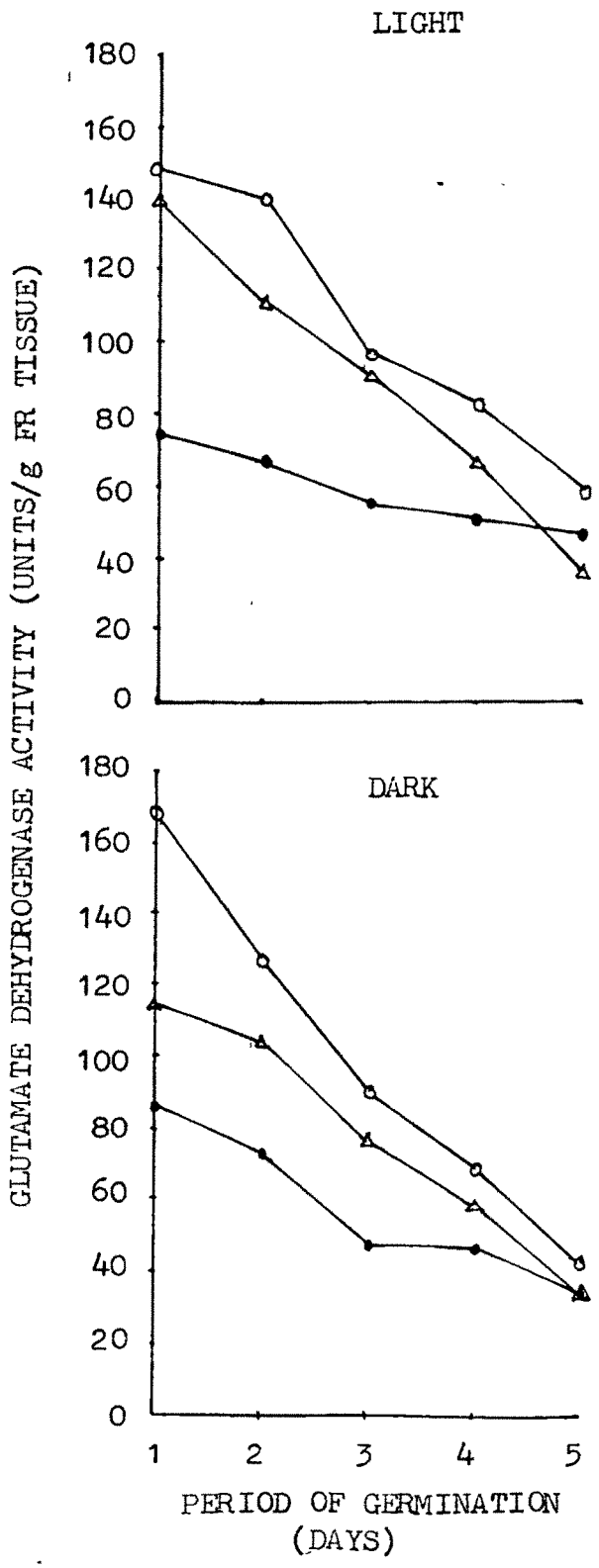

Fig. 5 : Effect of nitrogen sources on glutamate dehydrogenase activity during germination of radish seeds.
Control, ●—● ; +KNO_3, △—△ ; +NH_4Cl, ○—○ .

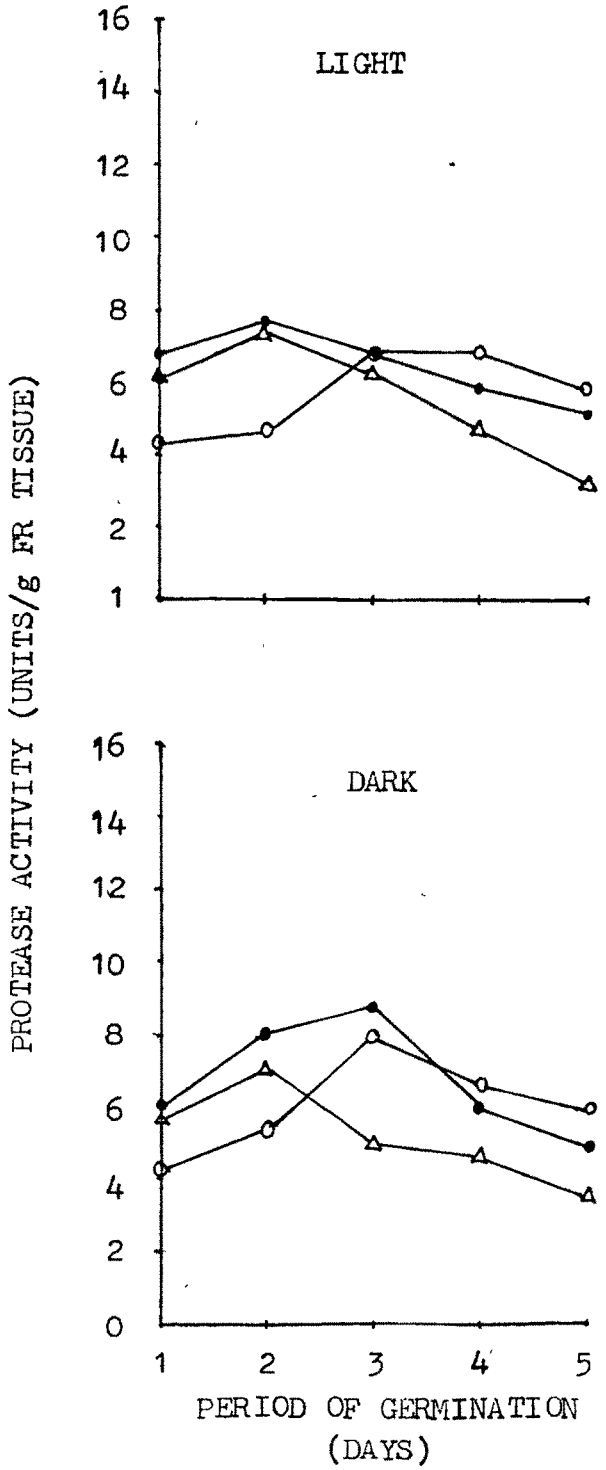

Fig. 6 : Effect of nitrogen sources on protease activity during germination of radish seeds.
Control, ●—● ; +KNO_3, △—△ ; +NH_4Cl, ○—○ .

control till the 2nd day of germination in light as well as dark but was increased to control level from the third day.

AAT activity increased with period of germination even in the absence of a nitrogen source (Fig. 7) in both light and dark grown seeds. The enzyme activity increased till the 4th day of germination and then declined. The supply of nitrate or ammonium had no effect on the level of the enzyme in dark grown seeds but a slightly lower activity was observed in light grown seeds during the later periods of germination.

Effect of polyamines and guanidines :

The effect of the compounds during germination of seeds was studied on the growth, level of nitrate assimilatory enzymes as well as protease and AAT in the absence or presence of a nitrogen source.

(a) Growth

In the absence of a nitrogen source (Table 1 and Fig.8) all the three amines tested brought about a 30-50% increase in the weight of the embryonal axis in light as well as dark grown seeds (Fig. 8 a,b). The polyamines, spermidine and spermine were slightly more effective than the diamine putrescine. Among the guanidines (Fig. 8 c,d), creatine

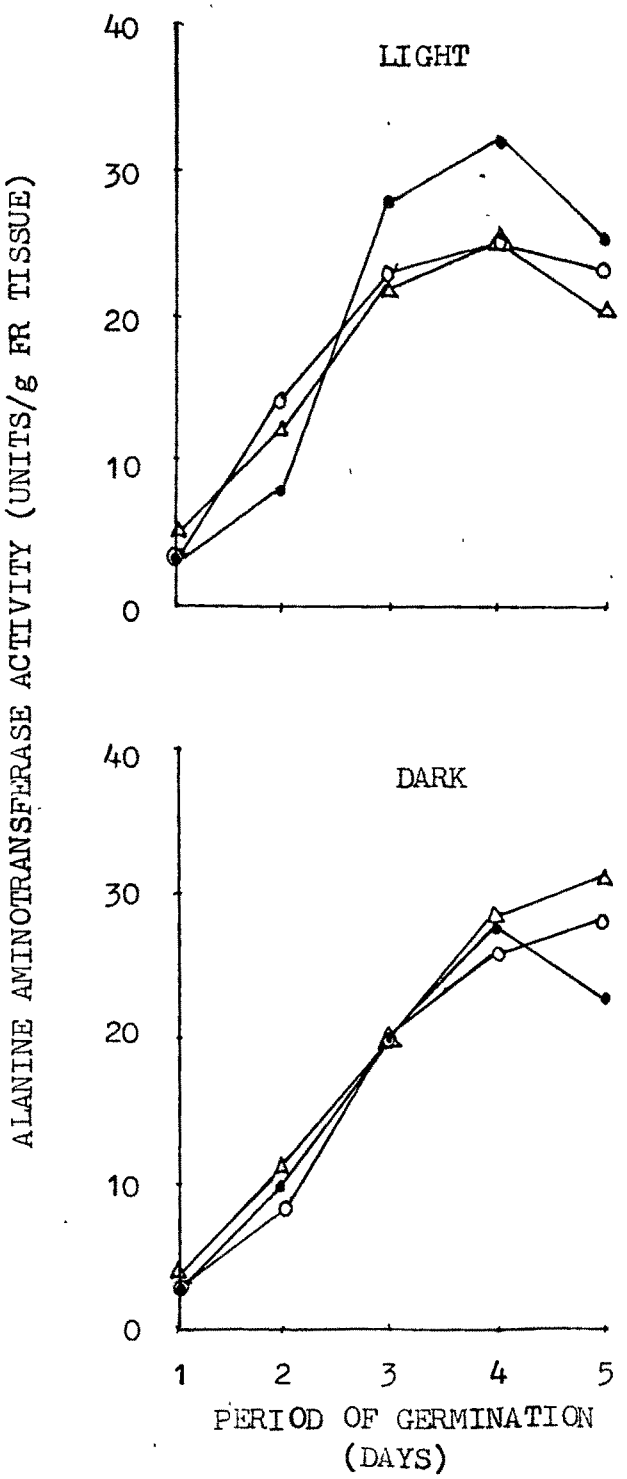

Fig. 7 : Effect of nitrogen sources on alanine aminotransferase activity during germination of radish seeds.
Control, ●——● ; +KNO_3, △——△ ; +NH_4Cl, o——o .

Table 1 : Effect of polyamines and guanidines on the growth of embryonal axis during germination of radish seeds in the absence of nitrogen source.

Treatment	Fresh weight (mg)/embryonal axis on day				
	1	2	3	4	5
			Light		
-	3	8	16	25	38
Putrescine	3	11	21	29	55
Spermidine	3	13	24	33	60
Spermine	3	12	26	35	63
Creatine	4	12	25	35	58
Dodine	3	12	17	23	45
GAA*	4	12	27	38	55
			Dark		
-	3	12	28	48	58
Putrescine	4	15	34	64	70
Spermidine	4	15	39	58	82
Spermine	4	16	39	64	85
Creatine	4	17	38	72	83
Dodine	3	16	29	55	63
GAA*	4	17	35	67	84

Seeds were germinated in continuous light or dark conditions without or with test compounds (1 mM). Embryos were excised and fresh weight recorded.

* GAA = Guanidino acetic acid

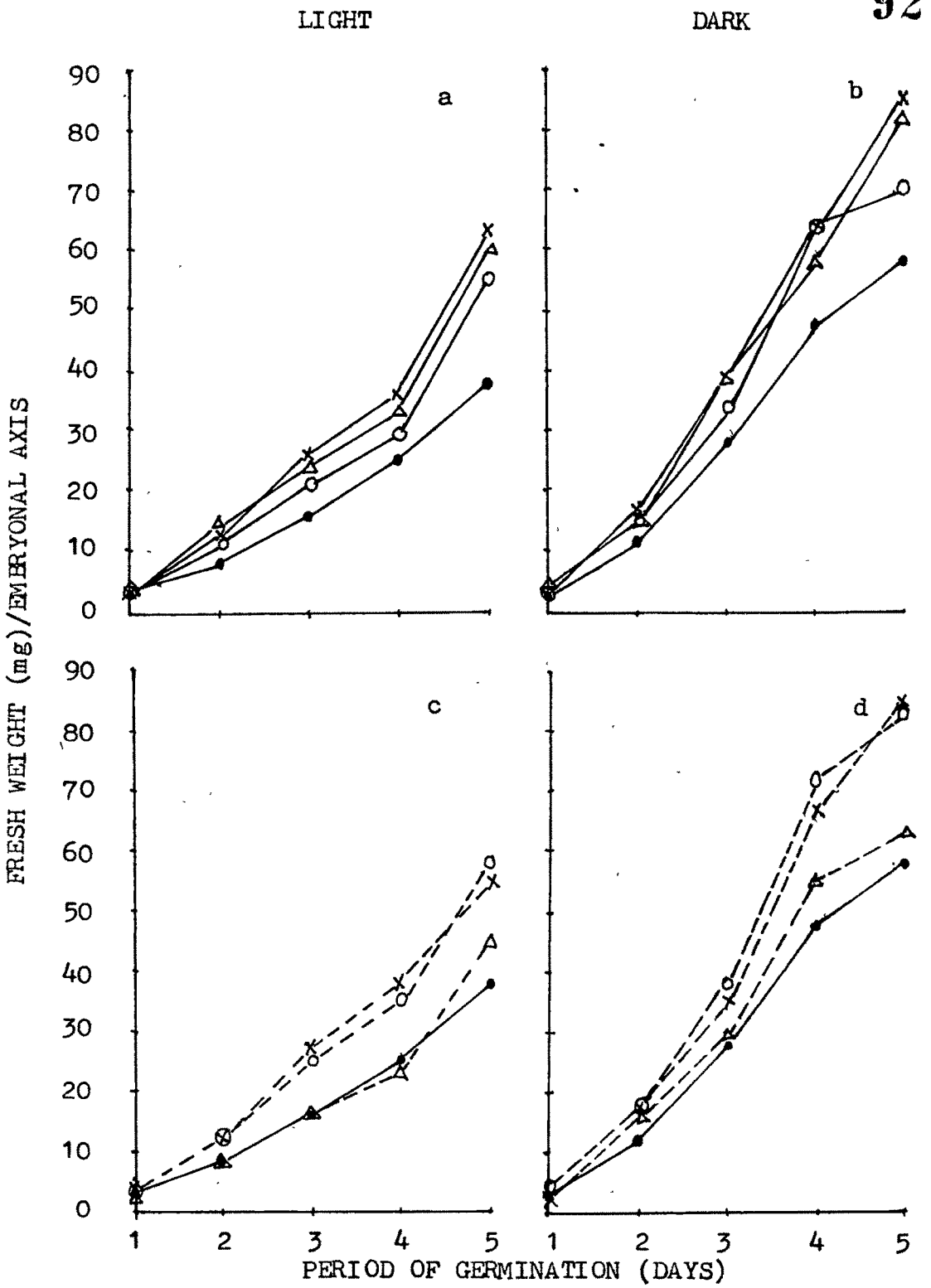

Fig. 8 : Effect of polyamines (a,b) and guanidines (c,d) on the growth of embryonal axis during germination of radish seeds in the absence of nitrogen source

Control, •—• ; putrescine, o—o ; spermidine, Δ—Δ ; spermine, ×—× ; creatine, o---o ; dodine, Δ----Δ ; GAA, ×----× .

and GAA caused an increase similar to polyamines in growth in both light and dark grown seeds. Dodine however, did not show any significant effect. The increase in growth by the compounds was evident from the 2nd day of germination and persisted throughout the period of germination.

A similar effect of amines and guanidines on growth was observed even when the seeds were grown in nitrate (Table 2 and Fig. 9). Earlier it was seen from Fig. 1 that nitrate itself brings about an increase in growth by about 30% over the control level and both amines and guanidines caused a further increase in growth by 30-50% over and above that obtained by nitrate alone. Ammonium grown seeds also showed a similar increase of 30-50% in growth (Table 3 and Fig. 10) in light grown seeds but dark grown seeds showed an increase only by the 5th day of germination.

(b) Enzymes

The effect of the compounds on the enzyme levels in the absence and presence of nitrogen sources is reported in Tables 4 to 21 and Figures 11 to 28.

In the absence of a nitrogen source neither polyamines nor guanidines showed any effect on NR activity (Table 4

Table 2 : Effect of polyamines and guanidines on the growth of embryonal axis during germination of radish seeds in presence of nitrate

Treatment	Fresh weight (mg)/embryonal axis on day				
	1	2	3	4	5
			Light		
-	2	8	18	39	51
Putrescine	3	10	23	46	58
Spermidine	3	12	27	49	66
Spermine	3	12	29	54	69
Creatine	3	13	31	49	66
Dodine	3	10	19	27	50
GAA	3	11	22	50	62
			Dark		
-	3	9	24	60	70
Putrescine	3	12	30	69	81
Spermidine	4	14	34	74	85
Spermine	3	14	38	77	88
Creatine	4	15	39	78	86
Dodine	3	12	31	51	70
GAA	4	13	40	73	86

Growth conditions were same as in Table 1 except that 10 mM KNO_3 was included in the growth medium.

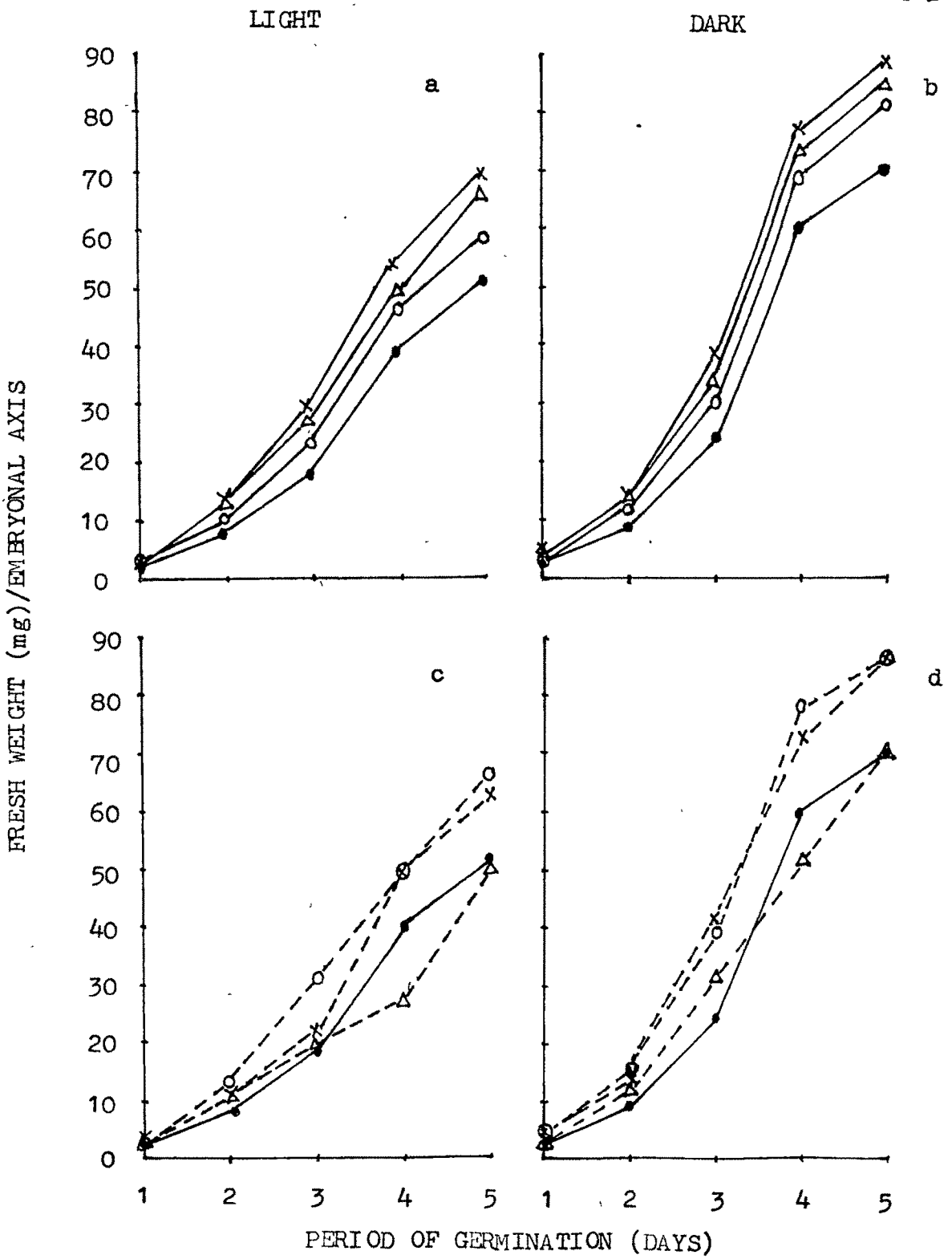

Fig. 9 : Effect of polyamines (a,b) and guanidines (c,d) on the growth of embryonal axis during germination of radish seeds in presence of nitrate.
Control, ●—● ; putrescine, o—o ; spermidine, Δ—Δ ; spermine, x—x ; creatine, o---o ; dodine, Δ---Δ ; GAA, x-x.

Table 3 : Effect of polyamines and guanidines on the growth of embryonal axis during germination of radish seeds in presence of ammonium

Treatment	Fresh weight (mg)/embryonal axis on day				
	1	2	3	4	5
			Light		
-	2	8	14	26	37
Putrescine	2	11	20	34	53
Spermidine	3	9	19	34	46
Spermine	2	11	21	38	54
Creatine	2	12	21	37	56
Dodine	3	9	12	20	29
GAA	3	9	18	35	51
			Dark		
-	2	6	16	37	40
Putrescine	3	9	19	41	57
Spermidine	3	8	20	41	50
Spermine	2	9	20	38	55
Creatine	3	10	18	48	55
Dodine	3	9	15	37	39
GAA	3	10	17	43	58

Growth conditions were same as in Table 1 except that 10 mM NH_4Cl was included in the growth medium.

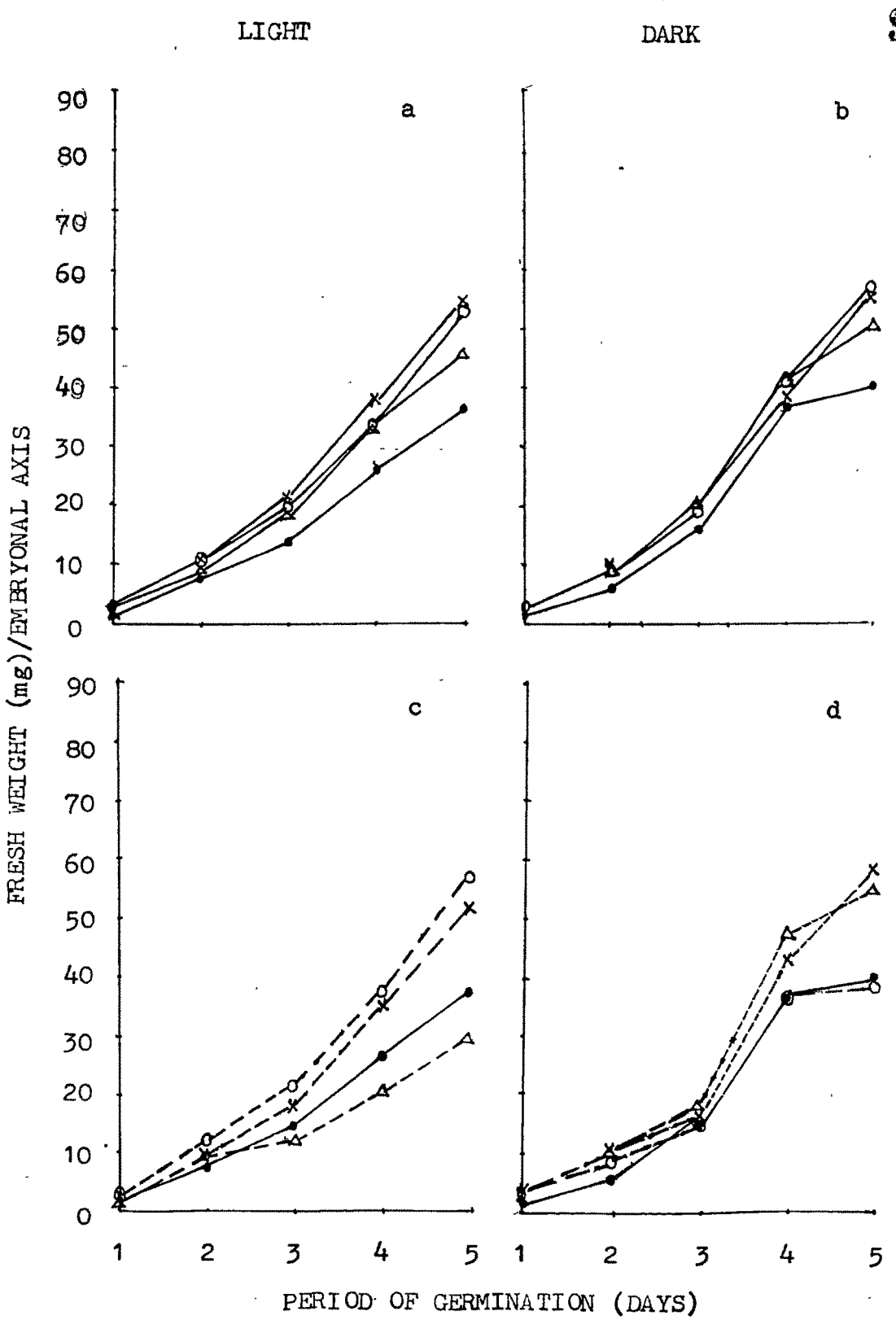

Fig. 10 : Effect of polyamines (a,b) and guanidines (c,d) on the growth of embryonal axis during germination of radish seeds in presence of ammonium.
Control, ●——● ; putrescine, o——o ; spermidine, △——△ ; spermine, ×——× ; creatine, o·····o ; dodine, △- - - -△; GAA, ×–×.

and Fig. 11) in light or dark grown seeds and the basal low level as in the case of control group was maintained. However, when the seeds were grown in the presence of nitrate, both polyamines and guanidines inhibited NR activity in light grown seeds (Table 5 and Fig. 12 a,c). The inhibitory effect was about 50% on the 2nd day of germination and decreased with decreasing activity during the period of germination. The compounds however, had no effect on the NR activity in dark grown seeds (Fig. 12 b,d). The compounds thus seem to affect only the light mediated increase in NR activity since the inhibited NR level was same as in the dark control group. In the presence of ammonium, however, (Table 6 and Fig. 13) polyamines and guanidines did not have any effect on the NR activity in light or dark grown seeds.

Polyamines and guanidines had no effect on NiR activity in light or dark grown seeds both in the absence and presence of nitrogen sources and the enzyme activity was almost same as in control groups (Tables 7,8,9 and Figs. 14,15,16).

The level of GS in seeds grown in the absence of a nitrogen source was not altered by polyamines and guanidines in light or dark (Table 10 and Fig. 17). However, when the seeds were grown in the presence of

Table 4 : Effect of polyamines and guanidines on nitrate reductase activity during germination of radish seeds in the absence of nitrogen source

Treatment	Nitrate reductase activity (units/g fr tissue) on day				
	1	2	3	4	5
-			Light		
-	0.06	0.44	0.59	0.50	0.44
Putrescine	0.05	0.43	0.59	0.53	0.45
Spermidine	0.06	0.40	0.49	0.57	0.48
Spermine	0.05	0.43	0.49	0.52	0.34
Creatine	0.05	0.28	0.51	0.53	0.54
Dodine	0.04	0.30	0.43	0.53	0.36
GAA	0.04	0.42	0.53	0.42	0.40
			Dark		
-	0.04	0.32	0.33	0.23	0.19
Putrescine	0.04	0.31	0.34	0.22	0.20
Spermidine	0.03	0.32	0.34	0.27	0.14
Spermine	0.02	0.37	0.30	0.30	0.17
Creatine	0.05	0.32	0.33	0.23	0.19
Dodine	0.04	0.22	0.32	0.21	0.16
GAA	0.06	0.21	0.36	0.28	0.22

Growth conditions were same as in Table 1.

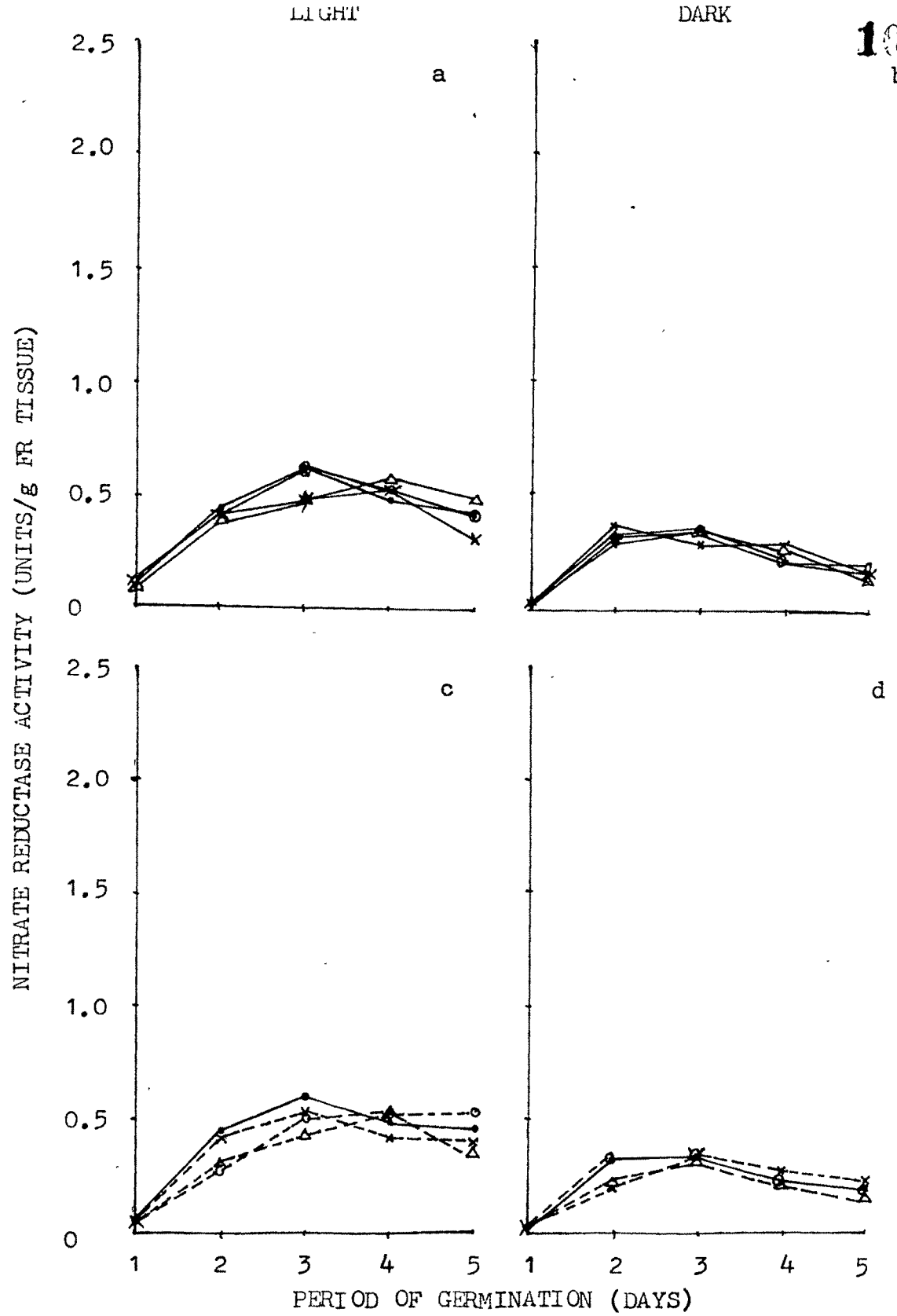

Fig. 11 : Effect of polyamines (a,b) and guanidines (c,d) on nitrate reductase activity during germination of radish seeds in absence of nitrogen sources. Control, ●—● ; putrescine, o—o ; spermidine, Δ—Δ ; spermine, ×—× ; creatine, o----o ; dodine, Δ---Δ ; GAA, ×---×.

Table 5 : Effect of polyamines and guanidines on nitrate reductase activity during germination of radish seeds in presence of nitrate

Treatment	Nitrate reductase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	0.34	2.33	1.72	0.86	0.49
Putrescine	0.31	1.28	1.24	0.77	0.34
Spermidine	0.32	1.22	1.28	0.79	0.42
Spermine	0.25	1.13	1.16	0.69	0.28
Creatine	0.33	1.15	1.02	0.86	0.49
Dodine	0.27	1.13	0.97	0.64	0.36
GAA	0.28	1.15	1.07	0.78	0.32
			Dark		
-	0.18	1.25	1.16	0.51	0.29
Putrescine	0.20	1.18	1.18	0.53	0.32
Spermidine	0.16	1.28	1.08	0.50	0.30
Spermine	0.18	1.18	1.09	0.49	0.23
Creatine	0.18	1.25	0.16	0.51	0.29
Dodine	0.14	1.19	0.84	0.46	0.27
GAA	0.18	1.30	0.90	0.49	0.26

Growth conditions were same as in Table 2.

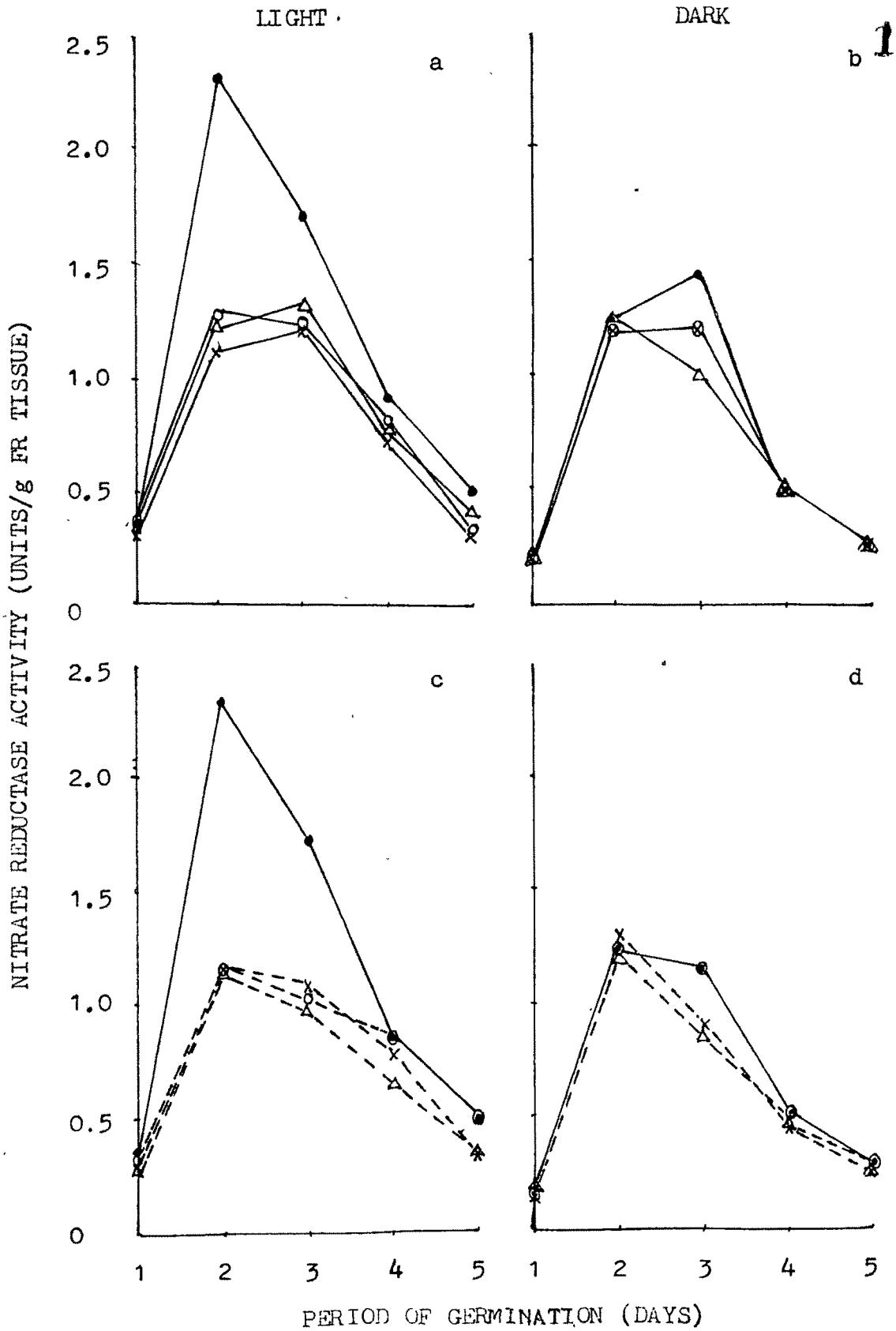

Fig. 12 : Effect of polyamines (a,b) and guanidines (c,d) on nitrate reductase activity during germination of radish seeds in presence of nitrate.
Control, ●——● ; putrescine, o——o ; spermidine, △——△ ; spermine, ×——× ; creatine, o----o ; dodine, △----△ ; GAA, ✱-✱.

Table 6 : Effect of polyamines and guanidines on nitrate reductase activity during germination of radish seeds in presence of ammonium

Treatment	Nitrate reductase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	0.18	0.52	0.78	0.60	0.32
Putrescine	0.20	0.45	0.80	0.46	0.22
Spermidine	0.20	0.29	0.73	0.44	0.21
Spermine	0.18	0.30	0.73	0.45	0.20
Creatine	0.17	0.34	0.51	0.54	0.33
Dodine	0.17	0.23	0.74	0.42	0.24
GAA	0.11	0.36	0.69	0.55	0.37
			Dark		
-	0.11	0.29	0.66	0.44	0.26
Putrescine	0.10	0.30	0.70	0.45	0.30
Spermidine	0.10	0.23	0.73	0.48	0.27
Spermine	0.08	0.30	0.71	0.43	0.26
Creatine	0.08	0.33	0.67	0.40	0.28
Dodine	0.07	0.29	0.69	0.46	0.27
GAA	0.09	0.30	0.65	0.53	0.30

Growth conditions were same as in Table 3.

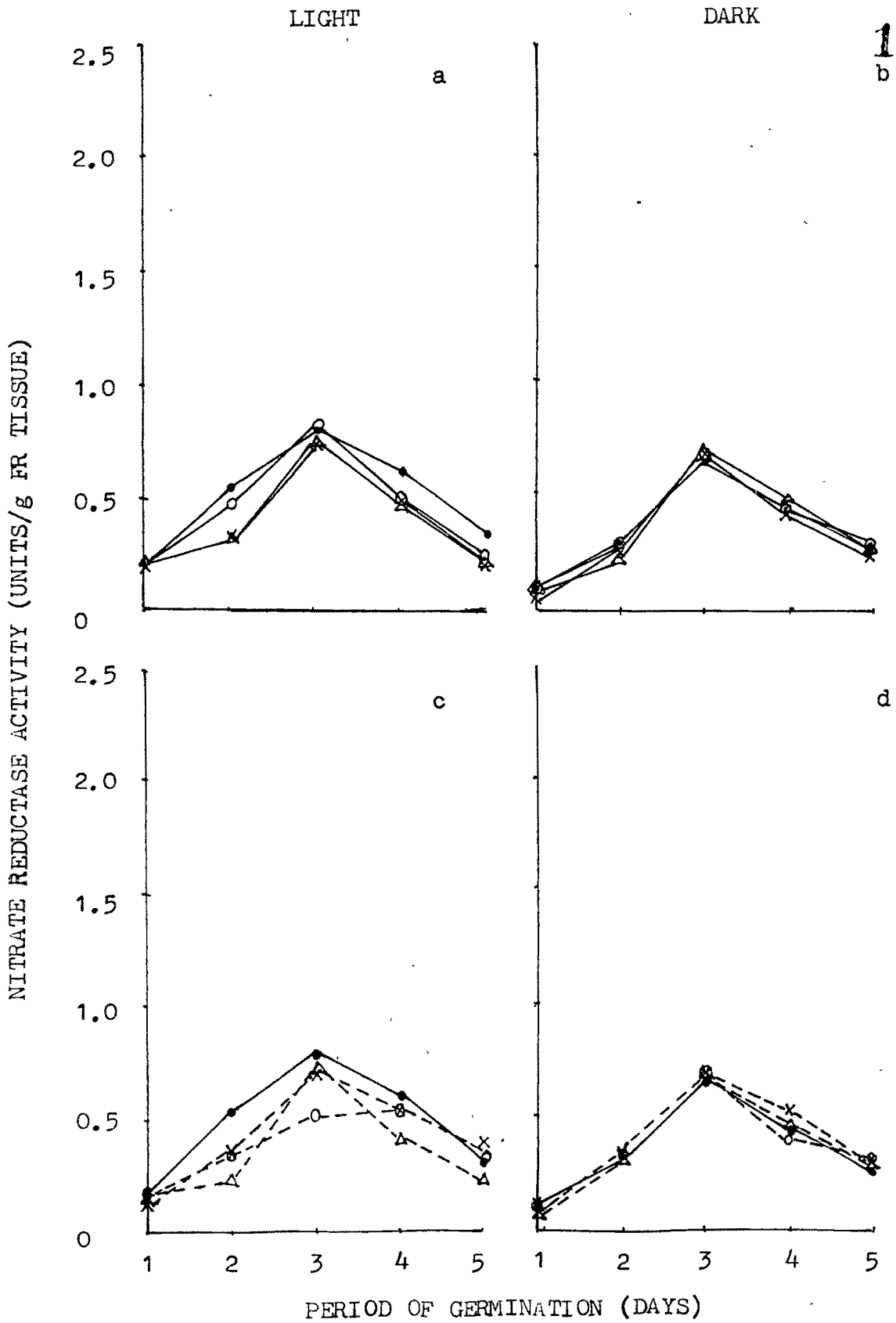

Fig. 13 : Effect of polyamines (a,b) and guanidines (c,d) on nitrate reductase activity during germination of radish seeds in presence of ammonium.
Control, ●——● ; putrescine, o——o ; spermidine, Δ——Δ ;
spermine, ×——× ; creatine, o- - - -o ; dodine, Δ- - - -Δ ; GAA, ×- -×.

Table 7 : Effect of polyamines and guanidines on nitrite reductase activity during germination of radish seeds in the absence of nitrogen source

Treatment	Nitrite reductase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	5	11	12	8	6
Putrescine	5	12	12	8	6
Spermidine	6	11	12	9	5
Spermine	5	10	10	8	6
Creatine	5	12	10	8	5
Dodine	5	12	11	9	5
GAA	5	12	10	10	5
			Dark		
-	5	10	11	7	6
Putrescine	4	9	9	8	6
Spermidine	5	9	10	8	5
Spermine	5	10	10	8	6
Creatine	4	10	10	7	6
Dodine	4	9	11	6	5
GAA	5	11	11	7	6

Growth conditions were same as in Table 1.

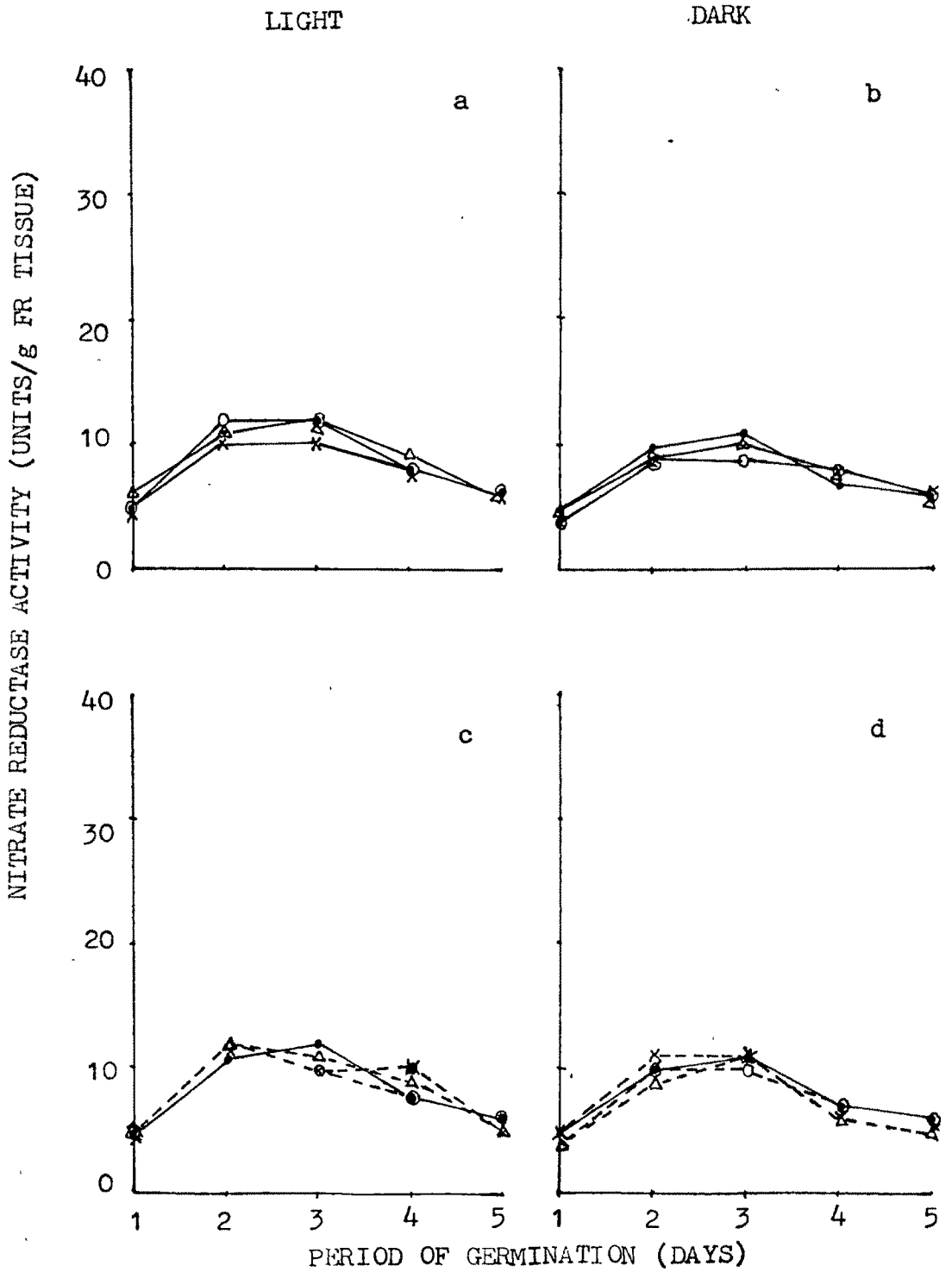

Fig. 14 : Effect of polyamines (a,b) and guanidines (c,d) on nitrite reductase during germination of radish seeds in the absence of nitrogen source.
Control, ●——● ; putrescine, o——o ; spermidine, △——△ ; spermine, ×——× ; creatine, o----o ; dodine, △----△ ; GAA, ×--×.

Table 8 : Effect of polyamines and guanidines on nitrite reductase activity during germination of radish seeds in presence of nitrate

Treatment	Nitrite reductase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	9	20	36	27	22
Putrescine	9	21	30	26	19
Spermidine	11	20	36	27	21
Spermine	9	23	33	23	19
Creatine	11	19	31	27	24
Dodine	8	15	30	25	20
GAA	12	23	36	29	22
			Dark		
-	9	13	19	23	19
Putrescine	9	13	18	22	18
Spermidine	9	14	17	21	19
Spermine	8	12	17	22	17
Creatine	9	11	19	22	20
Dodine	8	12	18	21	18
GAA	9	13	17	23	18

Growth conditions were same as in Table 2

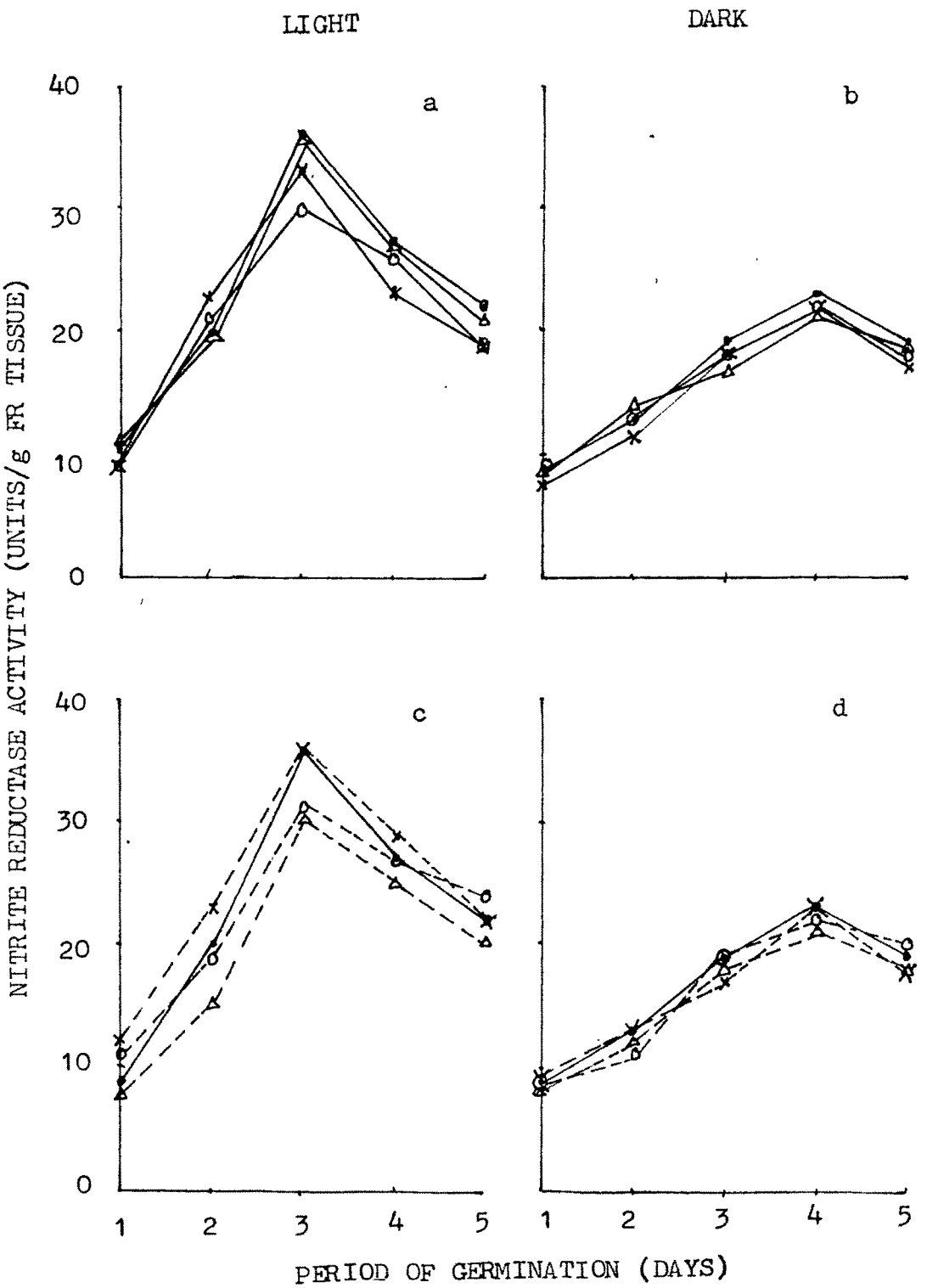

Fig. 15 : Effect of polyamines (a,b) and guanidines (c,d) on nitrite reductase activity during germination of radish seeds in presence of nitrate.
Control, ●——● ; putrescine, o——o ; spermidine, Δ——Δ ; spermine, ×——× ; creatine, o----o; dodine, Δ---Δ ; GAA, ×-×.

Table 9 : Effect of polyamines and guanidines on nitrite reductase activity during germination of radish seeds in presence of ammonium

Treatment	Nitrite reductase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	3	8	17	13	9
Putrescine	2	8	17	14	10
Spermidine	2	9	17	13	9
Spermine	2	8	18	13	9
Creatine	3	7	17	14	10
Dodine	2	8	16	13	10
GAA	3	8	20	14	10
			Dark		
-	2	6	11	8	7
Putrescine	2	7	10	9	9
Spermidine	2	7	11	8	8
Spermine	2	8	9	8	7
Creatine	2	7	9	7	8
Dodine	3	6	11	7	8
GAA	2	6	10	8	8

Growth conditions were same as in Table 3

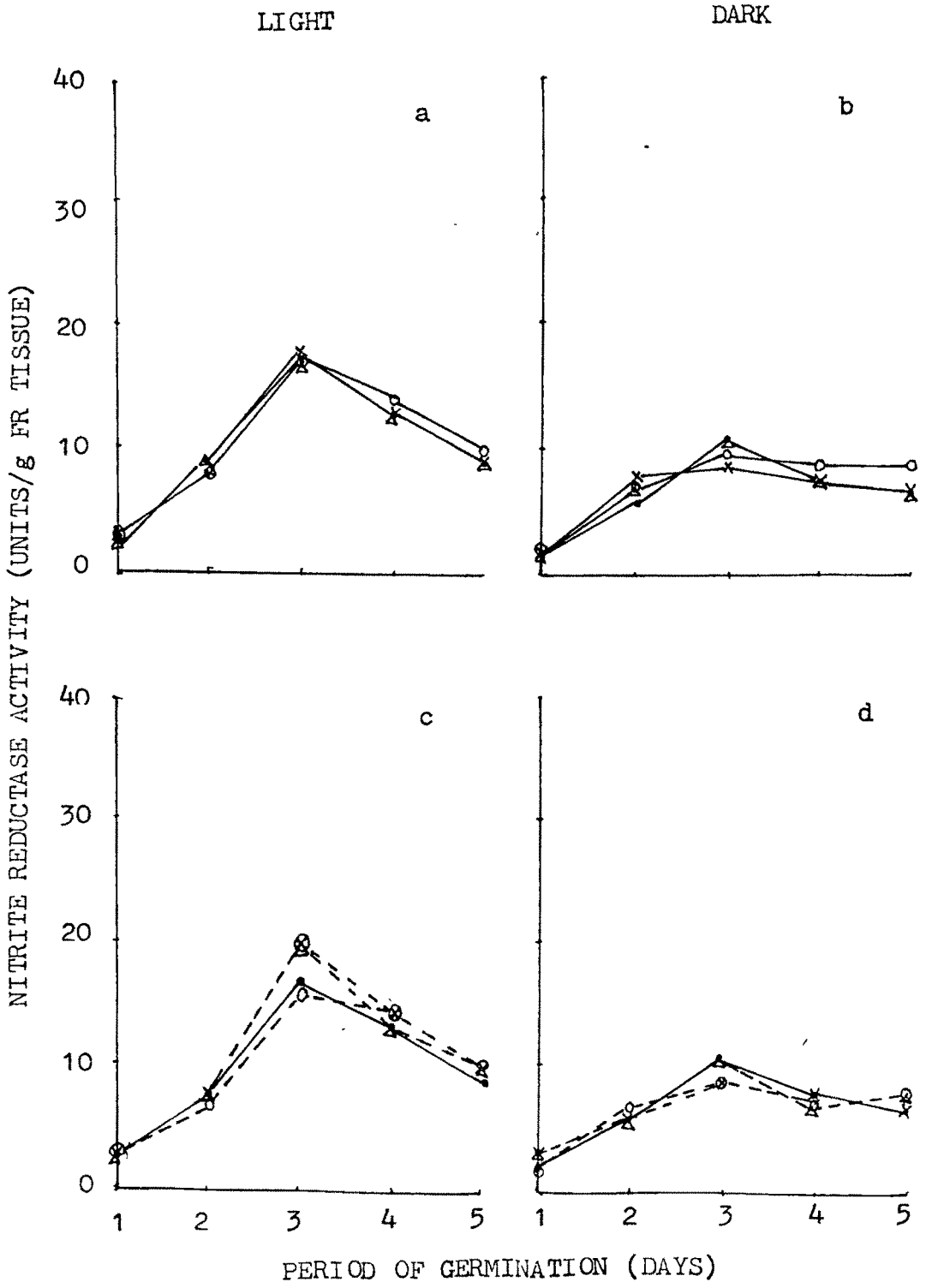

Fig. 16 : Effect of polyamines (a,b) and guanidines on nitrite reductase activity during germination of radish seeds in presence of ammonium.
Control, ●——● ; putrescine, o——o ; spermidine, △——△ ; spermine, ×——× ; creatine, o---o ; dodine, △---△ ; GAA, ×-×.

Table 10 : Effect of polyamines and guanidines on glutamine synthetase activity during germination of radish seeds in the absence of nitrogen source

Treatment	Glutamine synthetase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	5	7	10	8	5
Putrescine	6	7	10	9	6
Spermidine	7	6	9	8	5
Spermine	5	6	11	8	5
Creatine	5	8	10	8	5
Dodine	6	6	9	7	4
GAA	5	8	11	8	5
			Dark		
-	5	7	9	9	4
Putrescine	5	8	8	8	4
Spermidine	6	9	10	10	4
Spermine	6	8	9	10	5
Creatine	6	8	10	10	4
Dodine	5	8	10	9	4
GAA	5	9	9	8	5

Growth conditions were same as in Table 1

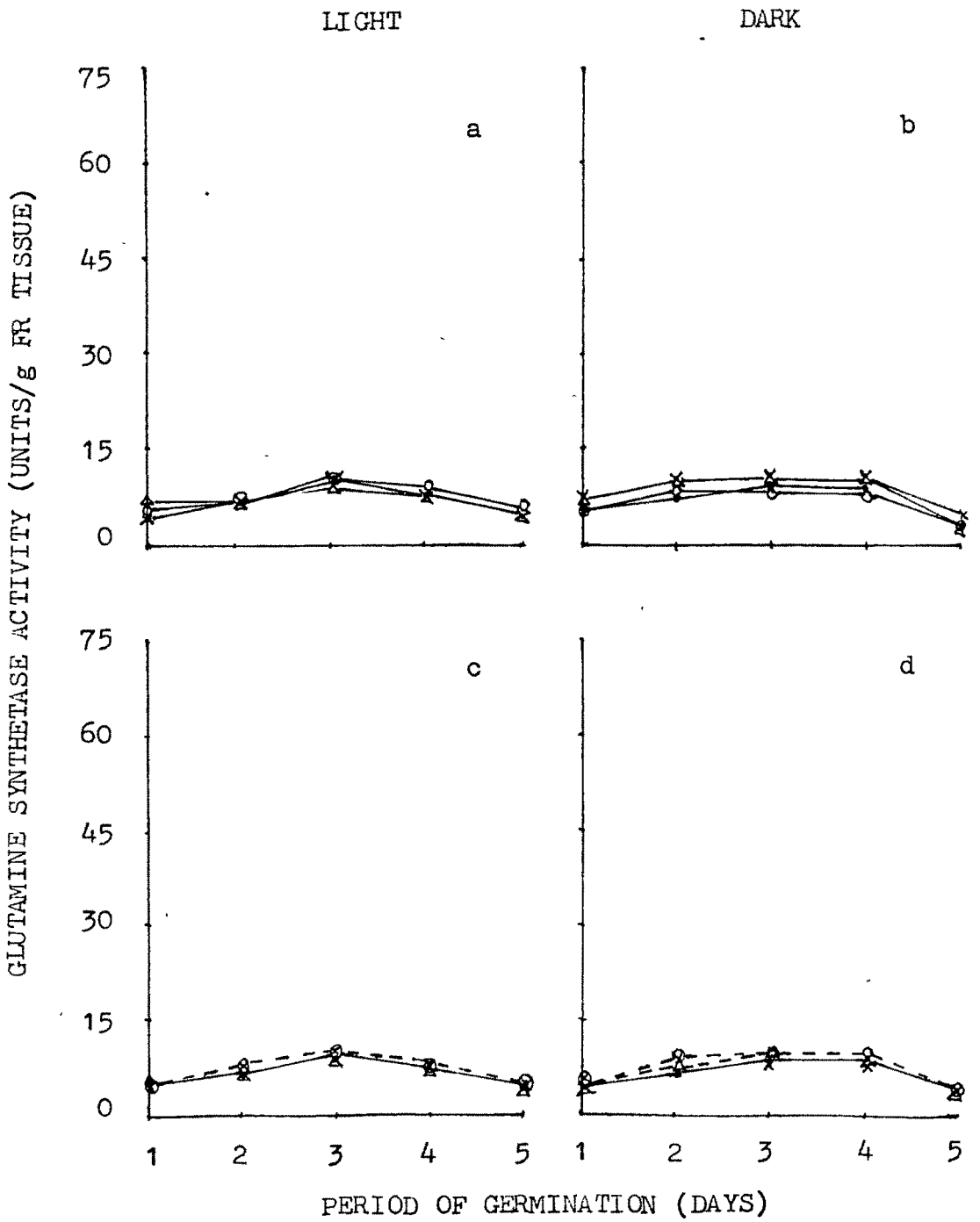

Fig. 17 : Effect of polyamines (a,b) and guanidines (c,d) on glutamine synthetase activity during germination of radish seeds in absence of nitrogen source.
Control, ●——● ; putrescine, o——o ; spermidine, Δ——Δ ; spermine, x——x ; creatine, o----o ; dodine, Δ---Δ ; GAA, x--x.

nitrate the GS activity in the light grown seeds was inhibited by about 35-40% by both polyamines and guanidines (Table 11 and Fig. 18 a,c) while that in the dark grown seeds (Table 11 and Fig. 18 b,d) was not affected. The inhibitory effect of the compounds in light persisted till the 4th day of germination. In the presence of ammonium both polyamines and guanidines did not have any effect on GS activity in light as well as dark grown seeds (Table 12 and Fig. 19).

The level of GDH was not affected by polyamines or guanidines whether the seeds were grown in the absence or presence of nitrogen sources (Tables 13,14,15 and Figs. 20,21,22) in light or dark conditions.

Polyamines as well as guanidines brought about a 20-50% increase in protease activity when the seeds were grown in the absence of a nitrogen source (Table 16 and Fig. 23). This effect was observed in both dark and light grown seeds. The activating effect was apparent from the first day of germination and persisted throughout the course of germination. The effect of the compounds was observed even when the seeds were grown in nitrate (Table 17 and Fig. 24) or ammonium (Table 18 and Fig. 25). In the presence of nitrate the effect of compounds was observed during the early period of germination which

Table 11 : Effect of polyamines and guanidines on glutamine synthetase activity during germination of radish seeds in presence of nitrate

Treatment	Glutamine synthetase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	8	30	70	29	12
Putrescine	7	22	62	17	10
Spermidine	7	19	55	15	10
Spermine	7	18	52	14	9
Creatine	8	22	60	20	11
Dodine	6	14	56	14	8
GAA	6	24	57	15	10
			Dark		
-	5	20	55	14	8
Putrescine	5	18	53	14	7
Spermidine	5	19	51	14	6
Spermine	4	17	53	13	6
Creatine	5	19	58	15	9
Dodine	4	17	51	13	6
GAA	6	21	54	13	7

Growth conditions were same as in Table 2

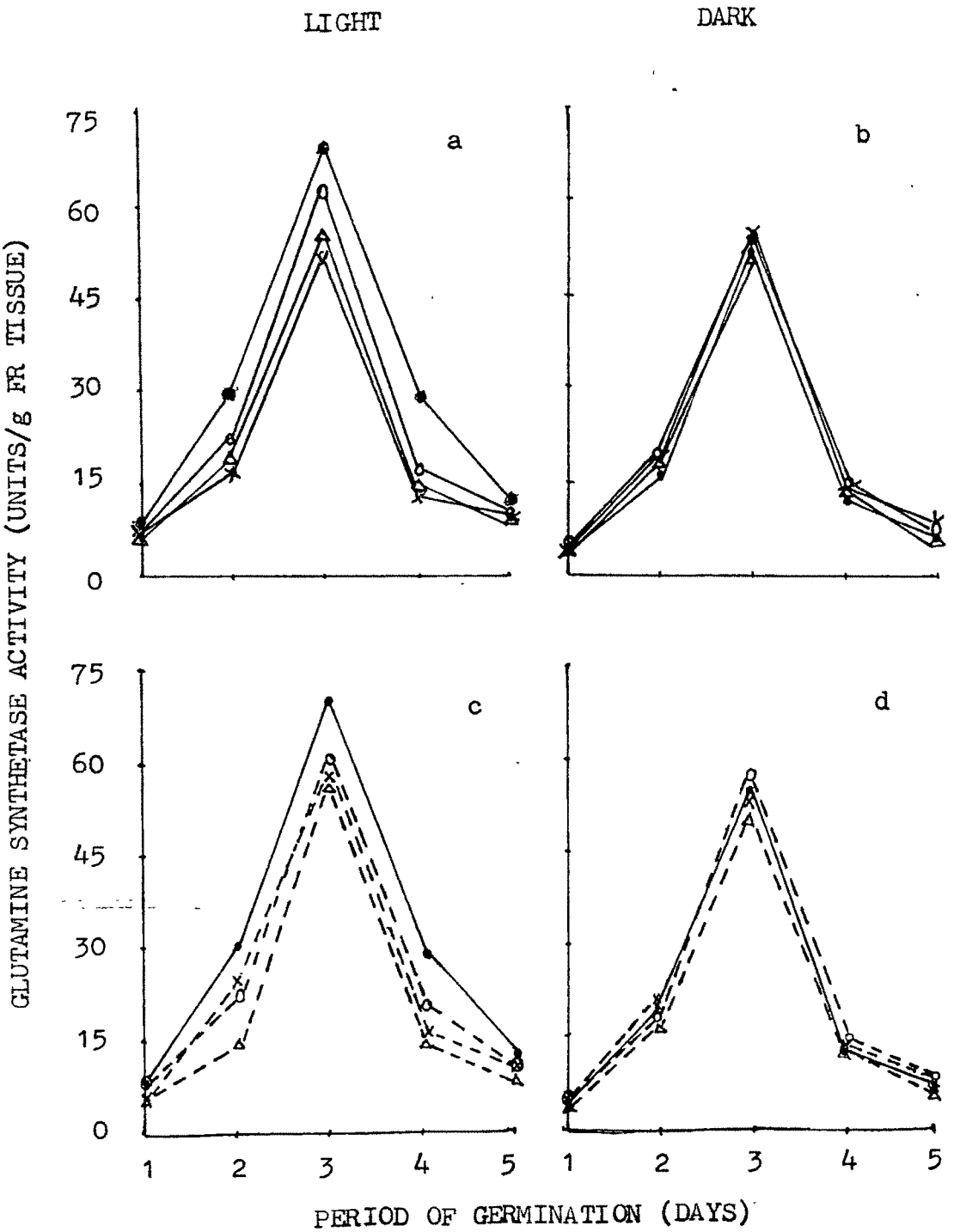

Fig. 18 : Effect of polyamines (a,b) and guanidines (c,d) on glutamine synthetase activity during germination of radish seeds in presence of nitrate.
Control, ●——● ; putrescine, o——o ; spermidine, △——△; spermine, ×——× ; creatine, o---o ; dodine, △---△ GAA, ×-×.

Table 12 : Effect of polyamines and guanidines on glutamine synthetase activity during germination of radish seeds in presence of ammonium

Treatment	Glutamine synthetase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	6	6	10	9	9
Putrescine	6	6	8	8	8
Spermidine	7	4	5	6	8
Spermine	6	5	8	8	7
Creatine	6	5	9	10	9
Dodine	6	9	8	9	8
GAA	6	5	9	10	10
			Dark		
-	3	5	9	7	5
Putrescine	2	5	9	7	6
Spermidine	2	4	8	8	6
Spermine	4	5	9	7	6
Creatine	3	5	10	8	5
Dodine	3	4	9	7	5
GAA	3	5	10	8	5

Growth conditions were same as in Table 3

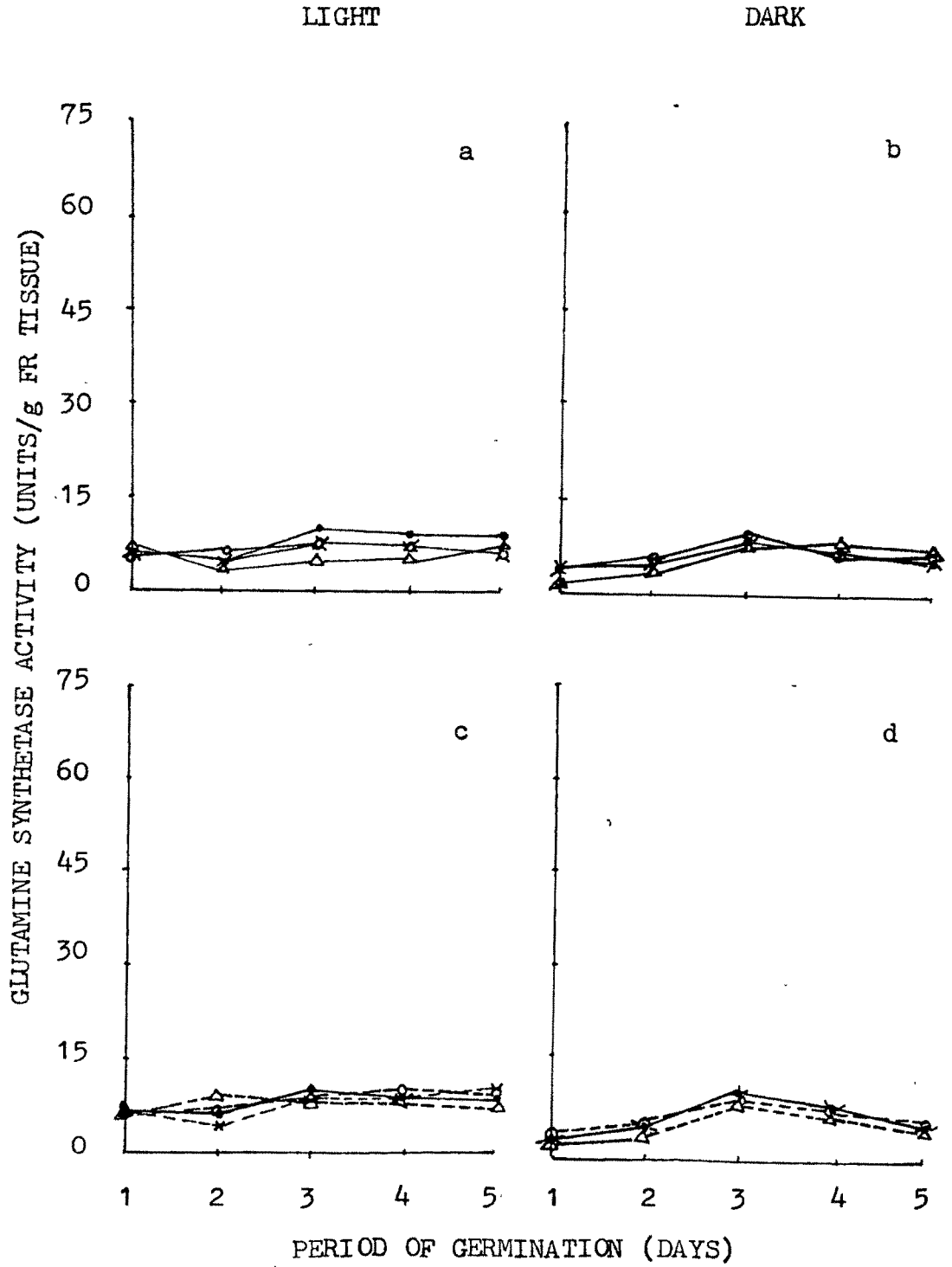

Fig. 19 : Effect of polyamines (a,b) and guanidines (c,d) on glutamine synthetase activity during germination of radish seeds in presence of ammonium.
Control, ●——● ; putrescine, o——o ; spermidine, Δ——Δ ; spermine, ×——× ; creatine, o- - -o ; dodine, Δ- - -Δ ; GAA, ×- -×

Table 13 : Effect of polyamines and guanidines on glutamate dehydrogenase activity during germination of radish seeds in the absence of nitrogen source

Treatment	Glutamate dehydrogenase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	74	67	55	50	46
Putrescine	81	63	51	46	42
Spermidine	73	64	55	51	38
Spermine	76	64	53	55	45
Creatine	80	69	56	53	44
Dodine	73	64	46	46	40
GAA	76	69	51	46	46
			Dark		
-	87	73	46	46	34
Putrescine	80	64	51	42	36
Spermidine	82	71	50	50	37
Spermine	89	75	51	47	34
Creatine	80	76	46	40	29
Dodine	76	73	56	46	38
GAA	84	74	44	51	46

Growth conditions were same as in Table 1

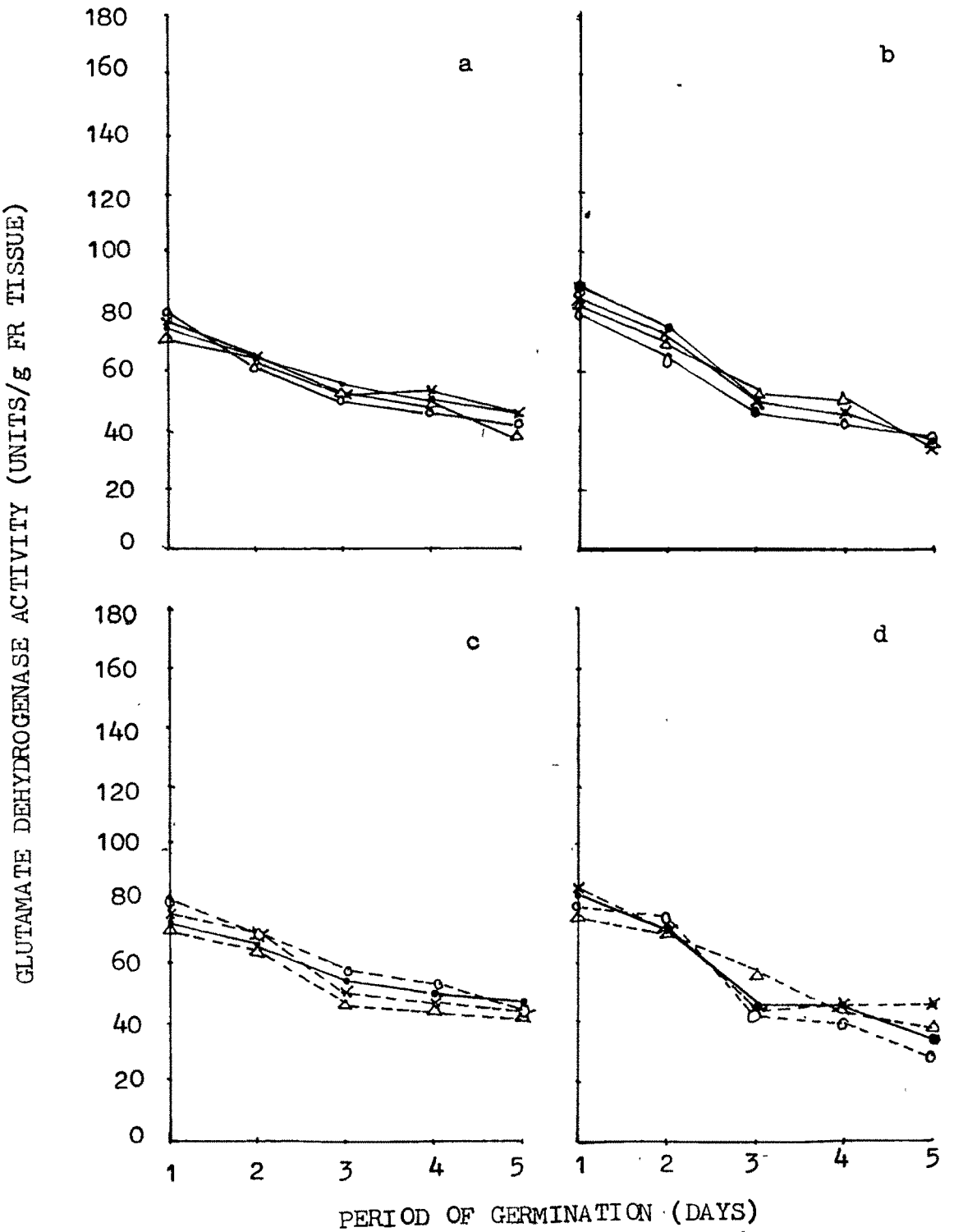

Fig. 20 : Effect of polyamines (a,b) and guanidines (c,d) on glutamate dehydrogenase during germination of radish seeds in absence of nitrogen source.
Control, ●——● ; putrescine, o——o ; spermidine, Δ——Δ ; spermine, X——X ; creatine, o----o ; dodine, Δ---Δ GAA, X---X.

Table 14 : Effect of polyamines and guanidines on glutamate dehydrogenase activity during germination of radish seeds in presence of nitrate

Treatment	Glutamate dehydrogenase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	139	110	90	66	36
Putrescine	140	110	90	72	32
Spermidine	140	114	78	66	36
Spermine	138	108	78	60	35
Creatine	134	104	84	72	27
Dodine	136	96	82	74	34
GAA	137	108	72	54	24
			Dark		
-	116	103	76	58	34
Putrescine	113	98	72	49	31
Spermidine	118	100	72	56	34
Spermine	114	91	68	48	37
Creatine	120	100	84	60	38
Dodine	108	97	68	52	36
GAA	126	99	74	58	30

Growth conditions were same as in Table 2

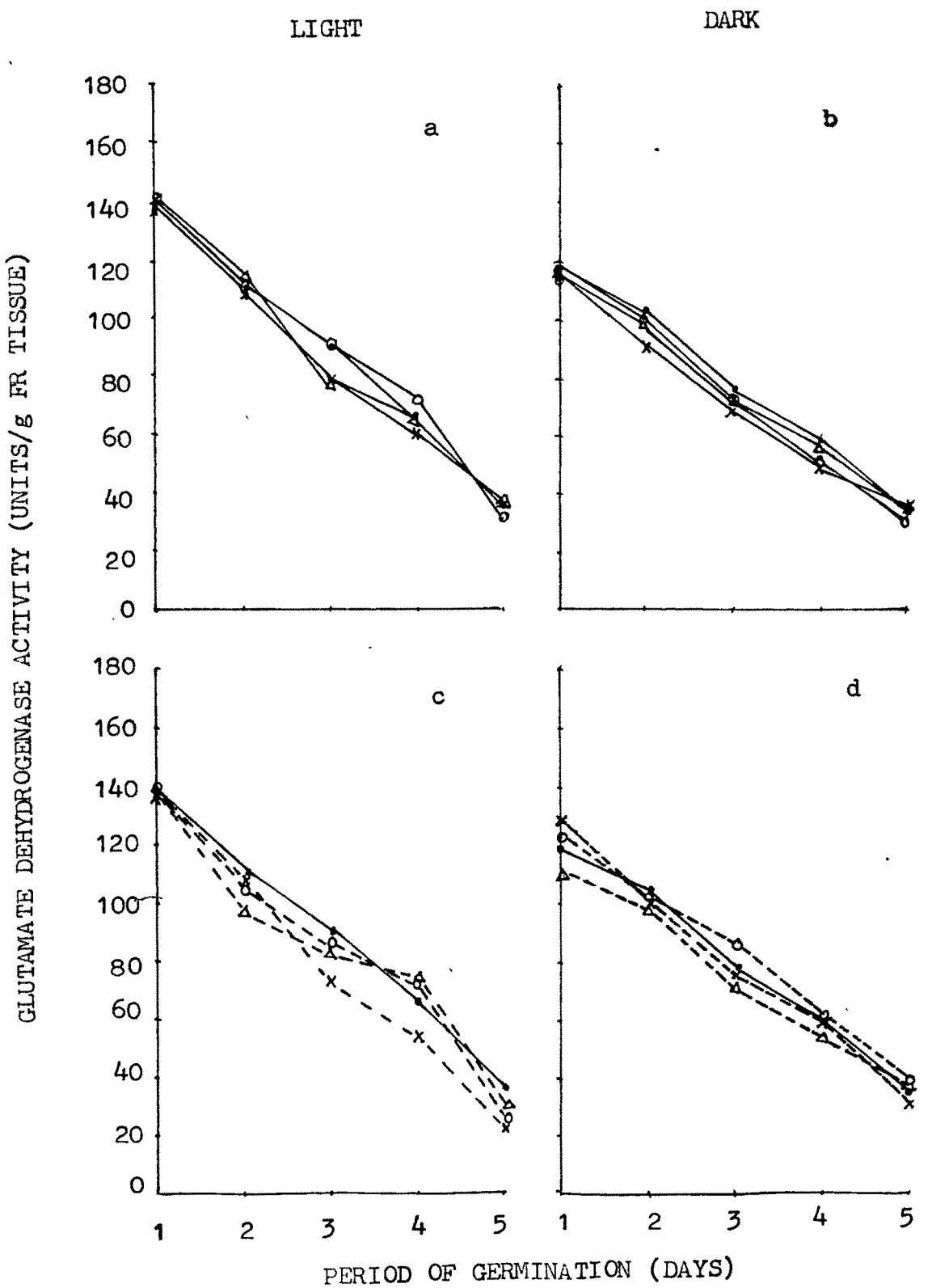

Fig. 21 : Effect of polyamines (a,b) and guanidines (c,d) on glutamate dehydrogenase activity during germination of radish seeds in presence of nitrate.
Control, •——• ; putrescine, o——o ; spermidine, Δ——Δ ; spermine, ×——× ; creatine, o---o ; dodine, Δ---Δ ; GAA, ×---×.

Table 15 : Effect of polyamines and guanidines on glutamate dehydrogenase activity during germination of radish seeds in presence of ammonium

Treatment	Glutamate dehydrogenase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	147	141	96	82	58
Putrescine	152	141	102	83	60
Spermidine	145	141	91	82	56
Spermine	145	140	98	90	55
Creatine	146	144	98	76	62
Dodine	147	145	96	82	60
GAA	140	145	100	83	63
			Dark		
-	167	127	89	69	43
Putrescine	176	119	82	71	45
Spermidine	168	116	88	64	50
Spermine	166	126	90	70	46
Creatine	171	125	101	71	39
Dodine	169	126	96	66	39
GAA	159	122	91	69	46

Growth conditions were same as in Table 3

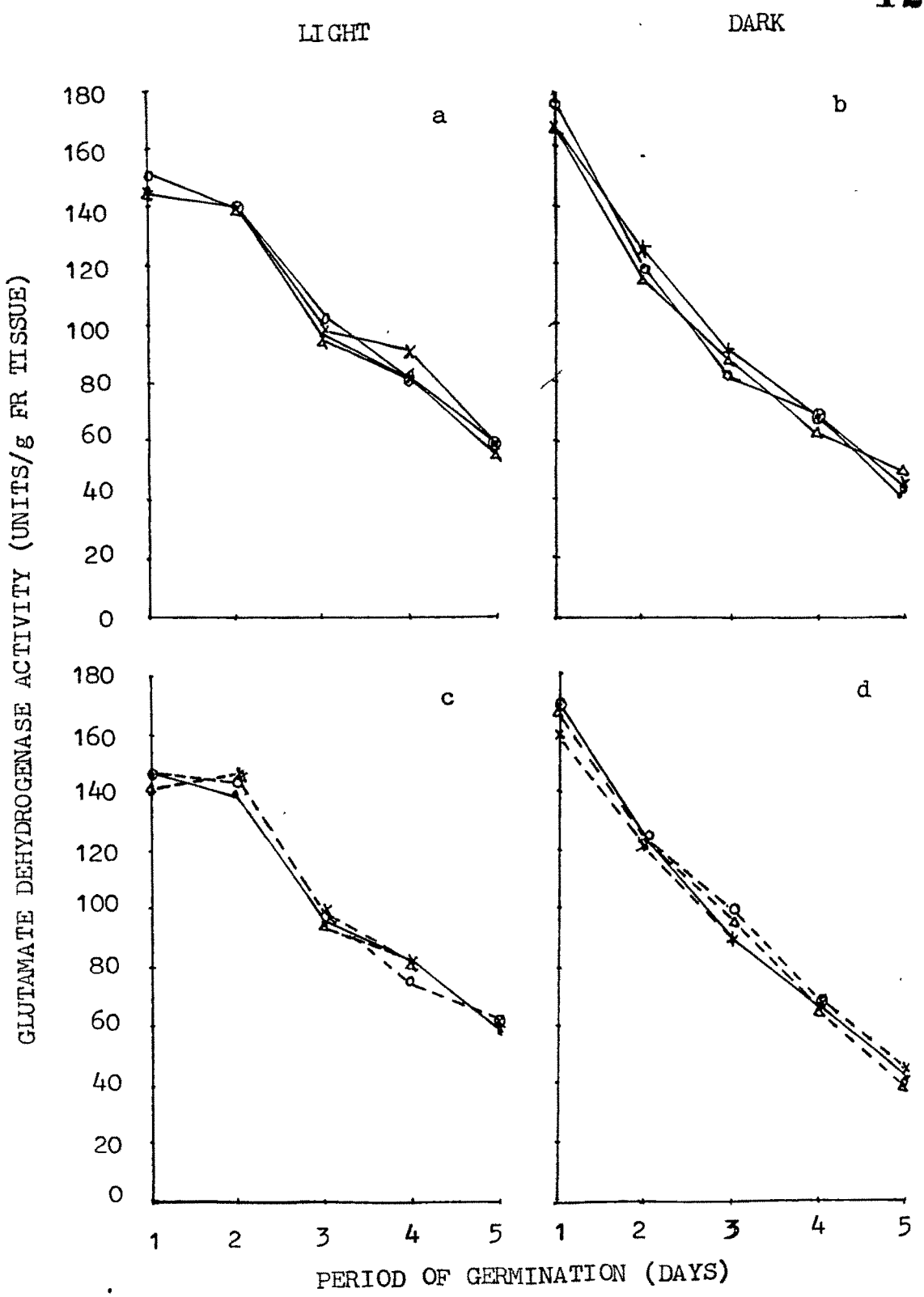

Fig. 22 : Effect of polyamines (a,b) and guanidines (c,d) on glutamate dehydrogenase activity during germination of radish seeds in presence of ammonium.
Control, ●——● ; putrescine, o——o ; spermidine, △——△ ; spermine, ×——× ; creatine, o---o ; dodine, △---△; GAA, ×-×.

Table 16 : Effect of polyamines and guanidines on protease activity during germination of radish seeds in absence of nitrogen source

Treatment	Protease activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	6.8	7.6	6.7	5.9	5.3
Putrescine	8.1	9.0	8.7	6.8	5.7
Spermidine	7.3	9.3	8.5	6.5	5.9
Spermine	7.3	9.4	8.4	6.8	6.9
Creatine	8.4	9.1	7.6	7.1	7.1
Dodine	7.6	8.8	8.1	6.9	6.1
GAA	8.5	7.9	8.8	6.7	6.2
			Dark		
-	6.0	8.0	8.8	6.0	5.0
Putrescine	6.8	8.6	8.9	7.6	6.6
Spermidine	6.9	8.9	11.3	7.8	6.8
Spermine	8.3	11.6	12.1	8.0	7.1
Creatine	6.9	8.8	9.6	9.0	6.9
Dodine	7.9	9.7	9.8	8.5	6.6
GAA	6.3	10.7	9.5	9.1	6.8

Growth conditions were same as in Table 1

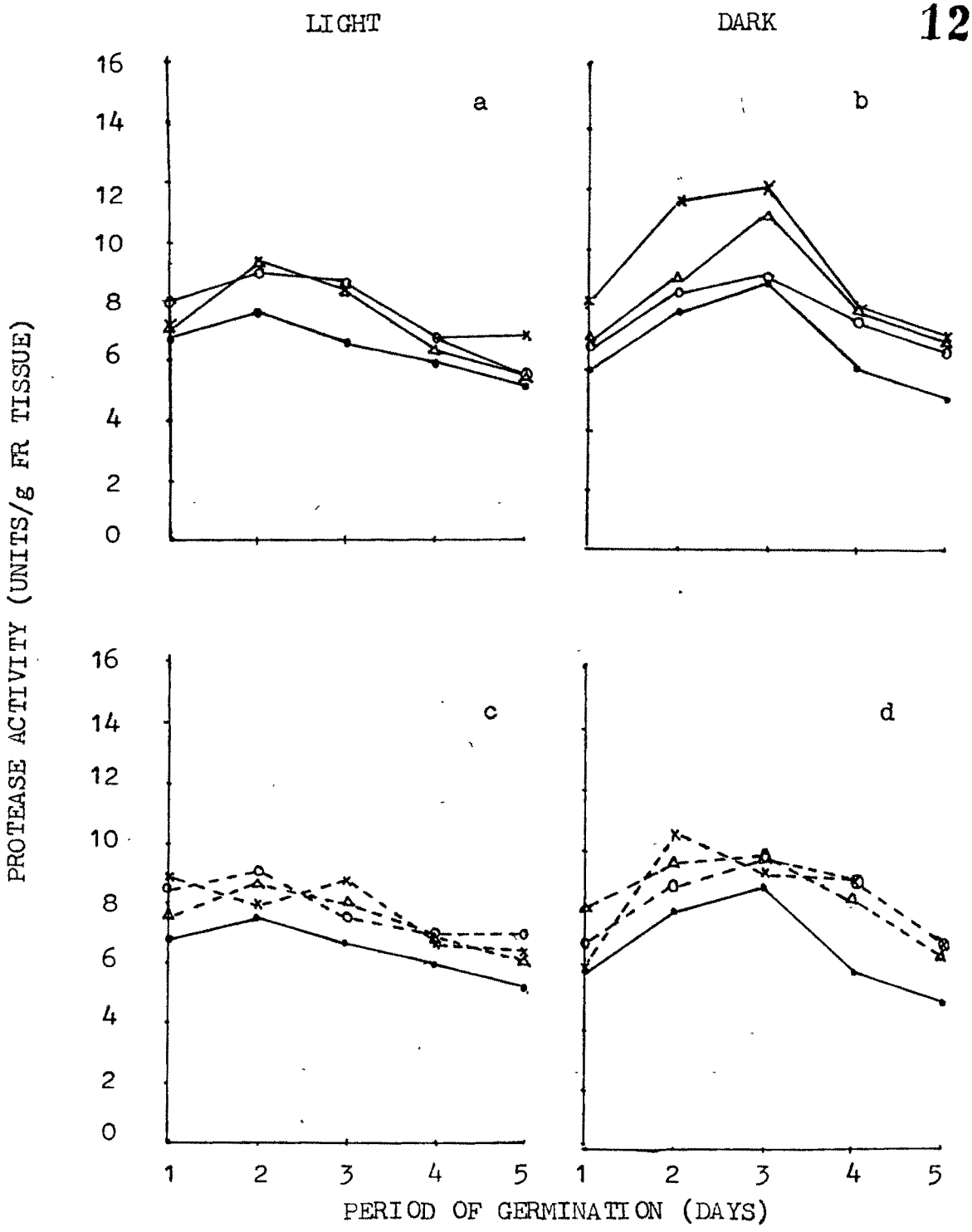

Fig. 23 : Effect of polyamines (a,b) and guanidines (c,d) on protease activity during germination of radish seeds in absence of nitrogen source.
Control, ●——● ; putrescine, o——o ; spermidine, Δ——Δ ; spermine, ✕——✕ ; creatine, o- - -o ; dodine, Δ- - -Δ ; GAA ✕- -✕.

Table 17 : Effect of polyamines and guanidines on protease activity during germination of radish seeds in presence of nitrate

Treatment	Protease activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	6.3	7.4	6.3	4.7	3.3
Putrescine	8.5	8.5	9.2	6.8	3.4
Spermidine	8.7	8.8	9.2	7.4	3.7
Spermine	8.6	10.5	9.2	7.8	4.4
Creatine	8.9	11.5	9.1	6.9	3.8
Dodine	7.7	9.7	9.0	6.5	3.6
GAA	8.4	9.8	8.3	6.9	3.6
			Dark		
-	5.8	7.1	5.3	4.5	3.6
Putrescine	7.0	8.6	7.7	5.3	4.4
Spermidine	8.0	9.8	8.8	5.6	5.0
Spermine	9.5	11.2	8.4	6.1	5.6
Creatine	8.3	10.4	9.2	7.1	6.0
Dodine	9.3	8.5	6.3	6.0	5.4
GAA	9.9	12.1	8.9	6.8	5.7

Growth conditions were same as in Table 2

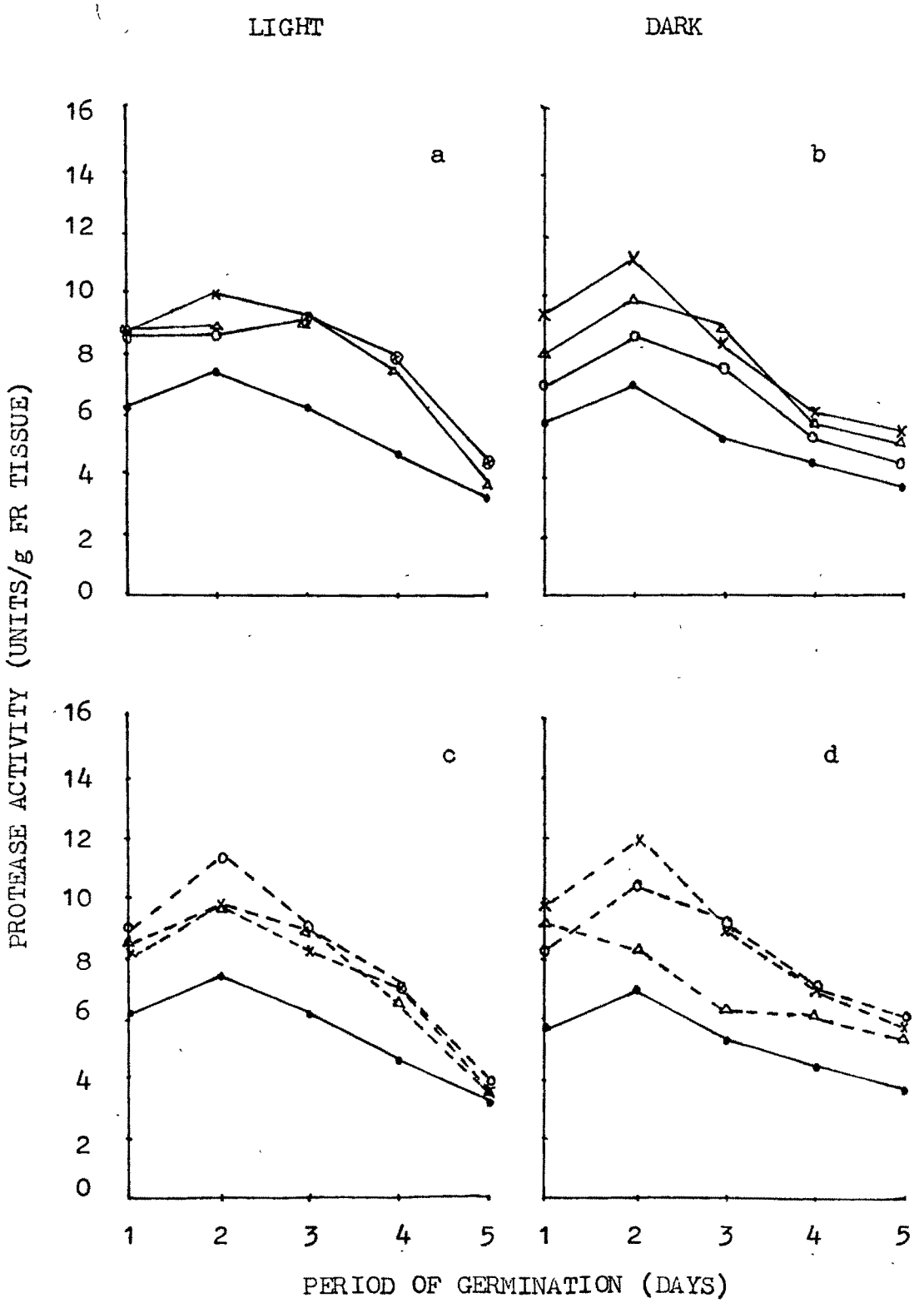

Fig. 24 : Effect of polyamines (a,b) and guanidines (c,d) on protease activity during germination of radish seeds in presence of nitrate.
Control, ●——● ; putrescine, o——o ; spermidine, △——△
spermine, ×——× ; creatine, o----o ; dodine, △----△GAA, ×-×.

Table 18 : Effect of polyamines and guanidines on protease activity during germination of radish seeds in presence of ammonium

Treatment	Protease activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	4.5	4.7	6.8	6.8	5.9
Putrescine	7.1	8.0	8.9	11.3	6.5
Spermidine	5.6	7.7	8.8	10.9	7.7
Spermine	5.5	7.8	9.7	11.5	7.3
Creatine	4.7	8.4	10.9	10.9	6.9
Dodine	4.3	8.7	9.9	9.4	6.4
GAA	6.8	7.8	9.0	9.9	6.7
			Dark		
-	4.4	5.4	8.0	6.5	6.0
Putrescine	6.4	7.7	10.1	12.4	8.6
Spermidine	6.7	7.9	11.4	12.6	10.6
Spermine	7.0	8.0	13.9	12.6	10.9
Creatine	8.2	7.7	9.1	14.6	10.6
Dodine	6.9	7.1	8.8	11.4	9.9
GAA	7.0	7.4	9.1	14.8	9.7

Growth conditions were same as in Table 3

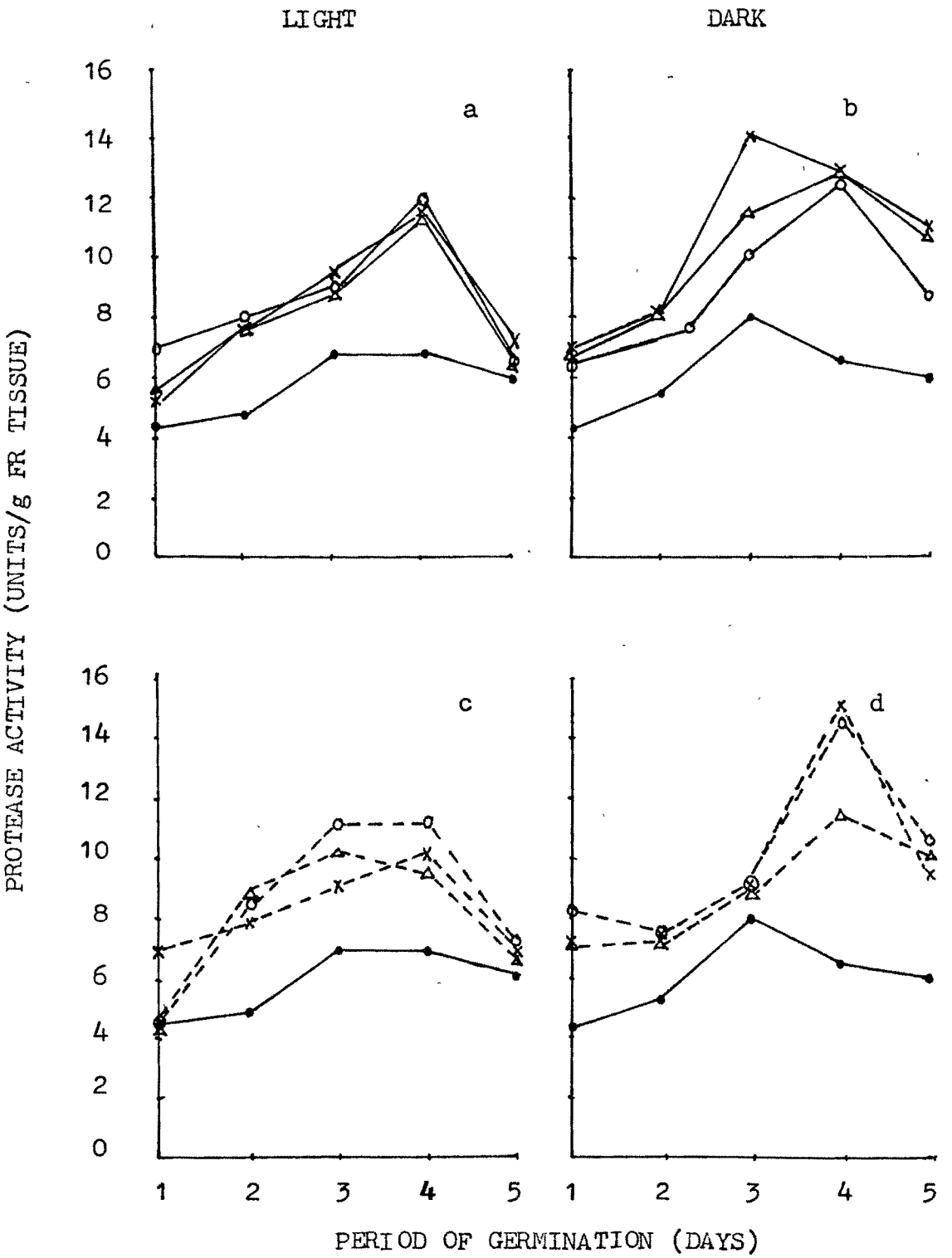

Fig. 25: Effect of polyamines (a,b) and guanidines (c,d) on protease activity during germination of radish seeds in presence of ammonium.
Control, ●—● ; putrescine, o—o ; spermidine, Δ—Δ ; spermine, ×—× ; creatine, o---o ; dodine Δ---Δ ; GAA, ×---×.

gradually declined to the control value by 5th day in light but remained higher in dark grown seedlings. However, in presence of ammonium the increase in protease activity by the compounds was observed during the later periods of germination. The activation decreased to control level on the 5th day in light but remained high in the dark grown ones.

Both the groups of compounds were also found to increase the activity of AAT by about 30-50% in light grown seeds (Table 19, Fig. 26 a,c) but had no effect in dark grown ones in the absence of a nitrogen source (Table 19 and Fig. 26 b,d). The effect of the compounds was observed from the 2nd day of germination. A similar increase of 30-40% in AAT activity of light grown seeds was observed in the presence of nitrate (Table 20 and Fig. 27 a,c) as well as ammonium (Table 21 and Fig. 28 a,c). In both the cases the enzyme in dark grown seeds was not affected.

These results indicated that when the seeds were grown in the presence of polyamines and guanidines the increase in growth by the compounds was associated with a decrease in NR and GS activity while the activity of protease and AAT were increased and there was no effect on NiR and GDH. The decrease or increase of the enzymes by the compounds was not due to inhibition or activation of

Table 19 : Effect of polyamines and guanidines on alanine aminotransferase activity during germination of radish seeds in absence of nitrogen source

Treatment	Alanine aminotransferase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	3	8	28	32	25
Putrescine	2	10	34	39	32
Spermidine	4	12	36	40	35
Spermine	2	10	38	43	33
Creatine	3	16	38	40	31
Dodine	3	16	38	39	35
GAA	2	18	38	39	33
			Dark		
-	3	10	20	28	23
Putrescine	3	11	23	29	23
Spermidine	2	13	22	28	24
Spermine	2	13	21	31	23
Creatine	2	14	23	31	22
Dodine	3	12	21	30	20
GAA	2	13	21	26	22

Growth conditions were same as in Table 1

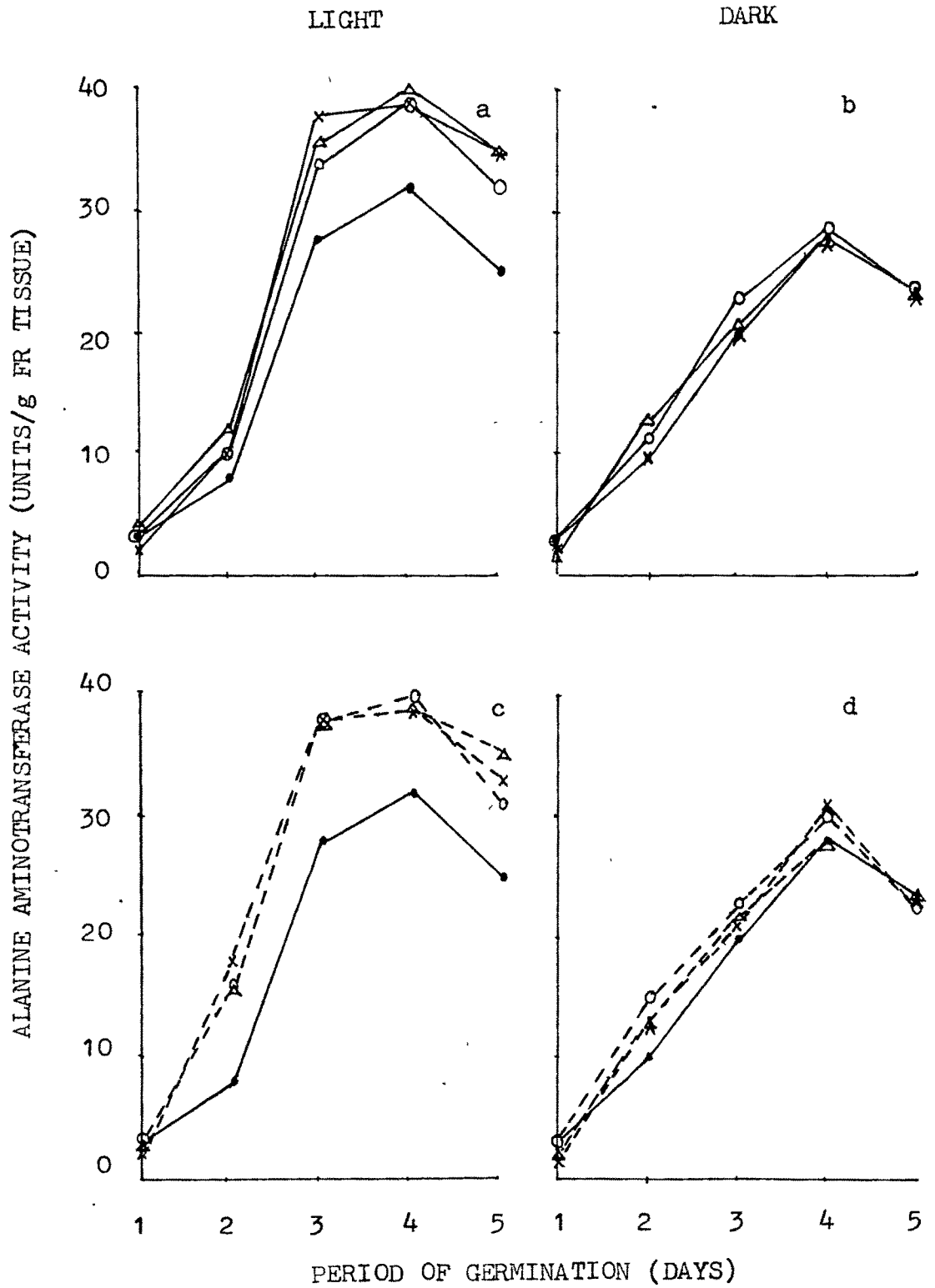

Fig. 26 : Effect of polyamines (a,b) and guanidines (c,d) on alanine aminotransferase activity during germination of radish seeds in absence of nitrogen source.
Control, ●—● ; putrescine, o—o ; spermidine, Δ—Δ ; spermine, ✕—✕ ; creatine, o---o ; dodine, Δ---Δ ; GAA, ✕---✕.

Table 20 : Effect of polyamines and guanidines on alanine aminotransferase activity during germination of radish seeds in presence of nitrate

Treatment	Alanine aminotransferase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	5	12	23	25	20
Putrescine	5	15	28	29	27
Spermidine	4	15	30	34	29
Spermine	4	16	32	38	29
Creatine	6	17	29	34	26
Dodine	5	16	26	35	28
GAA	6	16	30	29	26
			Dark		
-	4	11	20	29	33
Putrescine	3	11	21	31	33
Spermidine	4	12	21	29	34
Spermine	3	13	22	31	35
Creatine	3	12	22	33	36
Dodine	2	13	19	28	30
GAA	2	11	22	32	34

Growth conditions were same as in Table 2

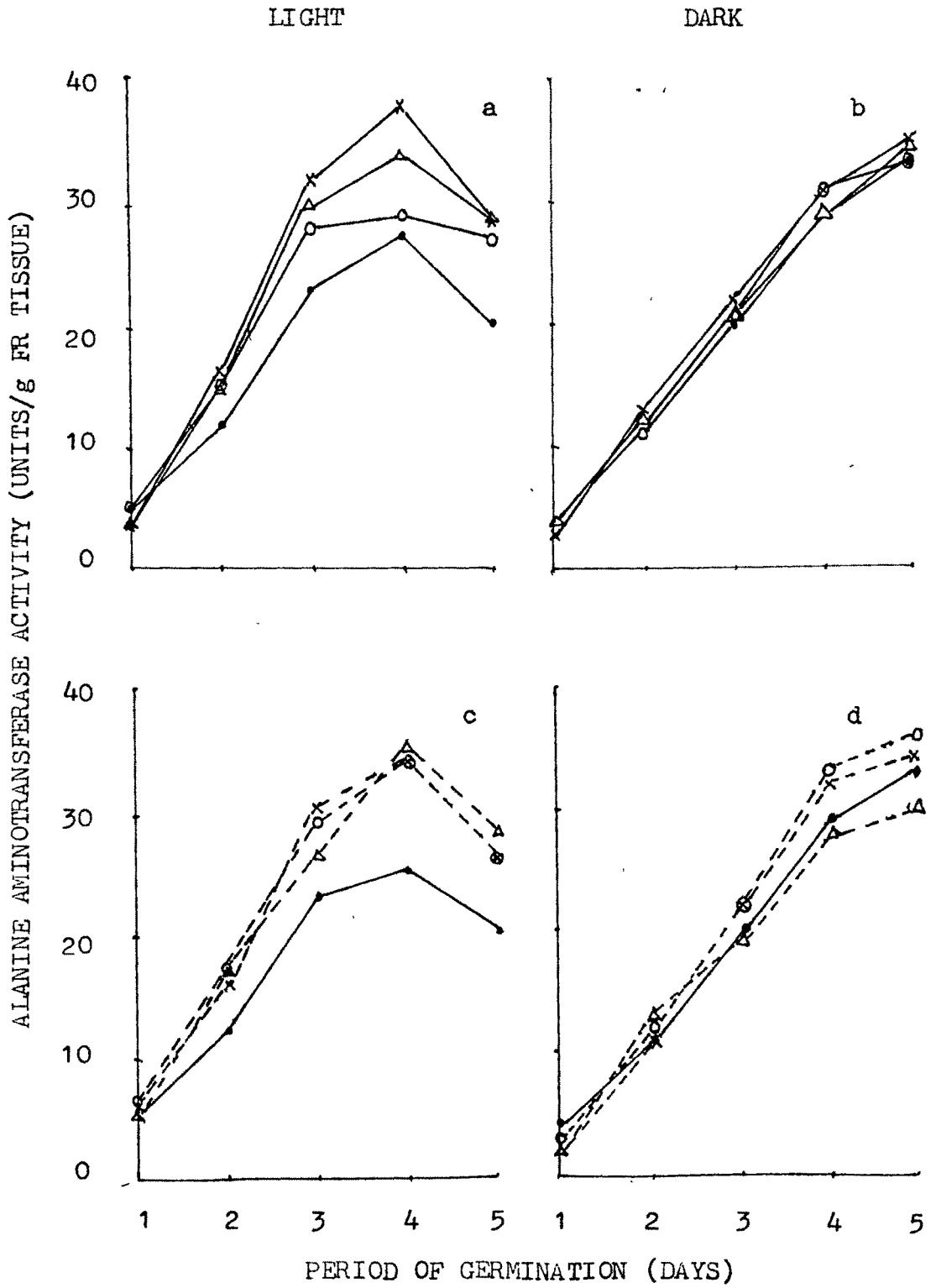

Fig. 27 : Effect of polyamines (a,b) and guanidines (c,d) on alanine aminotransferase activity during germination of radish seeds in presence of nitrate.
Control, ●——● ; putrescine, O——O ; spermidine, Δ——Δ ; spermine, ×——× ; creatine, o---o ; dodine, Δ---Δ ; GAA, ×--×.

Table 21 : Effect of polyamines and guanidines on alanine aminotransferase activity during germination of radish seeds in presence of ammonium

Treatment	Alanine aminotransferase activity (units/g fr tissue) on day				
	1	2	3	4	5
			Light		
-	3	14	22	25	23
Putrescine	2	15	26	29	31
Spermidine	3	15	28	29	32
Spermine	3	15	30	37	33
Creatine	4	18	28	32	37
Dodine	2	14	31	29	33
GAA	3	15	29	35	37
			Dark		
-	3	8	20	26	28
Putrescine	4	10	22	26	29
Spermidine	3	9	20	27	29
Spermine	3	9	21	26	31
Creatine	4	9	17	28	30
Dodine	3	7	17	26	25
GAA	2	9	18	26	29

Growth conditions were same as in Table 3

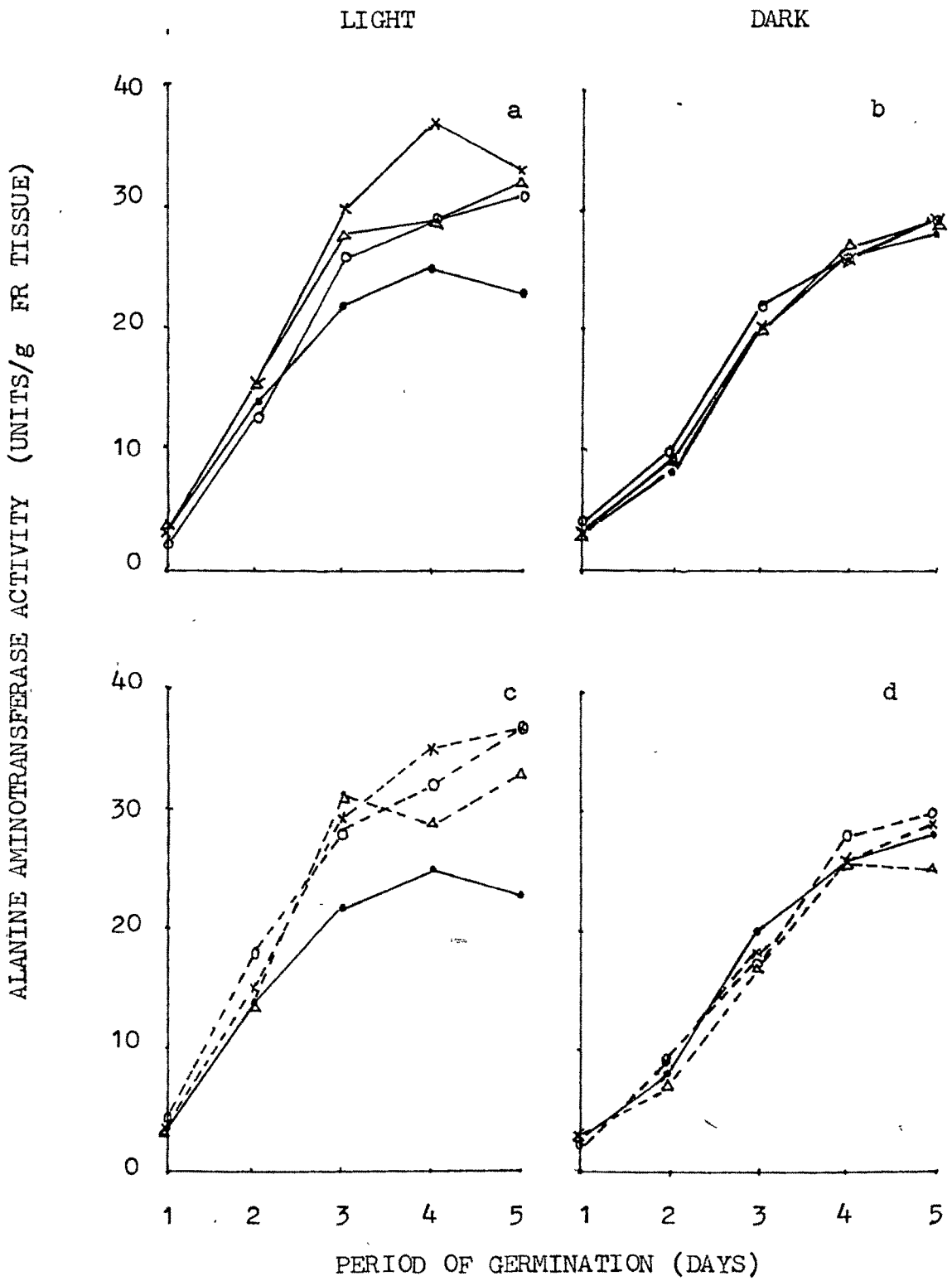

Fig. 28 : Effect of polyamines (a,b) and guanidines (c,d) on alanine aminotransferase activity during germination of radish seeds in presence of ammonium.
Control, ●—● ; putrescine, o—o ; spermine, Δ—Δ ; spermine, ×—× ; creatine, o---o ; dodine, Δ--Δ ; GAA, ×--×.

the enzyme activity since addition of these compounds during the assay of the enzymes had no effect (Table 22). Further studies were then carried out to investigate the mechanism of inhibition or activation of the enzymes. Since all the polyamines and guanidines showed a similar effect on the enzymes, the experiments reported hereafter were carried out with spermine and GAA as representative compounds of polyamines and guanidines respectively.

The level of NR is dependent upon the amount of nitrate available in the tissue. Since polyamines and guanidines are known to affect membrane permeability, the nitrate content of the tissue grown in the absence or presence of these compounds was measured. The compounds had no effect on the nitrate content of the tissues (Table 23).

Since NR is known to be induced in the excised tissues also, further studies to investigate the mechanisms of action of the compounds on NR were carried out during the in vitro induction using excised cotyledons. Seeds were germinated in the absence of any nitrogen source for different periods and NR activity was induced in the excised cotyledons. The NR activity was found to be induced in cotyledons from both light and dark grown seedlings and maximal level of enzyme was reached in

Table 22 : Effect of spermine and GAA on the enzymes when added in the assay system

Compound	Enzyme activity (units/g fr tissue)			
	Nitrate reductase	Glutamine synthetase	Protease	Alanine amino-transferase
-	1.8	72	5	20
Spermine	1.7	72	5	19
GAA	1.8	76	6	20

Seeds were germinated in continuous light in presence of KNO_3 (10 mM) for 72 hr.

Concentration of spermine and GAA was 1 mM.

Table 23 : Effect of spermine and GAA on nitrate content of cotyledons during germination of radish seeds in presence of nitrate

Period of Germination (days)	Nitrate (µmoles/ g fr tissue)		
	Control	+ Spermine	+ GAA
1	3.1	3.1	3.0
2	5.8	6.0	6.0
3	6.0	6.2	6.4

Seeds were germinated in continuous light without or with the test compounds (1 mM).

cotyledons grown for 48 hr (Table 24). However, 36 hr of germination was selected for further induction studies. The induction was found to be optimum at about 3 hr (Table 25), and at 10 mM nitrate concentration (Table 26). Very little increase in activity was observed when the period of induction was increased to 4-5 hr (Table 25) or nitrate concentration to 40 mM (Table 26).

The effect of polyamines and guanidines when studied in the excised cotyledons during the induction of NR activity in light or dark, showed (Table 27) that putrescine had no effect on the in vitro induction of NR activity in light or dark conditions. Both the polyamines, however, inhibited NR activity by about 40-70% in light and dark. Spermine was more effective than spermidine. This is in contrast to the earlier observations where polyamines inhibited only the light mediated increase in NR activity when the seeds were grown in the presence of nitrate (Table 5).

Among the guanidino compounds tested, creatine had no effect but GAA and dodine inhibited NR activity by 50-70% during induction in light or dark. Dodine was more effective than GAA.

The inhibitory effect of spermine was concentration dependent and increased to 75% at 5 mM (Table 28). Since

Table 24 : Induction of nitrate reductase activity in excised cotyledons of radish seeds

Period of germination (hr)	Nitrate reductase activity (units/g fr tissue) in	
	Light	Dark
24	0.72	0.72
28	1.01	0.62
36	1.62	0.96
48	1.90	1.24
72	1.13	0.26
96	0.65	0.46

Seeds were germinated in light or dark conditions in the absence of a nitrogen source. Cotyledons were excised at the specified periods and NR activity was induced with KNO_3 (10 mM) for 3 hr.

Table 25 : Effect of period of induction on nitrate reductase activity in excised cotyledons of radish seeds

Period of induction (hr)	Nitrate reductase activity (units/g fr tissue)
0.25	0.08
0.50	0.22
1.0	0.39
2.0	0.80
2.5	0.92
3.0	1.06
4.0	1.22
5.0	1.36

Seeds were germinated in dark for 36 hr in the absence of nitrogen source. Cotyledons were excised and NR activity was induced in dark with KNO_3 (10 mM). NR activity was assayed at specified periods of induction.

Table 26 : Effect of nitrate concentration on the induction of nitrate reductase activity in excised cotyledons of radish seeds

KNO_3 concentration (mM)	Nitrate reductase activity (units/g fr tissue)
2	0.14
5	0.54
10	0.94
20	1.22
40	1.24

Growth and induction conditions were same as in Table 25. Induction was carried out for 3 hr.

Table 27 : Effect of polyamines and guanidines on the induction of nitrate reductase activity in excised cotyledons of radish seeds

Pretreatment	Nitrate reductase activity (units/g fr tissue)	
	Light	Dark
-	1.83 (100)	1.19 (100)
Putrescine	1.80 (98)	1.13 (98)
Spermidine	1.19 (65)	0.71 (60)
Spermine	0.65 (36)	0.36 (30)
Creatine	1.76 (96)	1.08 (91)
Dodine	0.54 (30)	0.38 (32)
GAA	1.00 (55)	0.62 (52)

Seeds were germinated in dark for 36 hr in the absence of nitrogen source. Cotyledons were excised and pretreated with test compounds (1 mM) for 30 min before induction with KNO_3 (10 mM) in light or dark for 3 hr.

Figures in the parentheses represent per cent of control value taken as 100.

Table 28 : Effect of concentration of spermine on the induction of nitrate reductase activity in excised cotyledons of radish seeds

Spermine concentration (mM)	Nitrate reductase activity (units/g fr tissue)
-	0.83
0.5	0.68
1.0	0.38
2.0	0.40
5.0	0.22

Growth conditions were same as in Table 27.

Cotyledons were excised and pretreated with different concentrations of spermine for 30 min and induced in dark with KNO_3 (10 mM) for 3 hr.

polyamines and guanidines are known to affect membrane permeability and as NR activity is dependent upon the amount of nitrate available in the tissue their effect was also studied on the uptake of nitrate. The effect of spermine was studied on the uptake at high and low concentrations of ambient nitrate. However, the nitrate left over in the medium at the end of induction period did not differ in the absence or presence of spermine (Table 29) indicating that spermine does not affect the uptake of nitrate. Spermine also had no effect on the enzyme activity when added to the assay system in vitro (Table 30).

Though the compounds did not affect the uptake of nitrate they may have an effect on the intracellular distribution of nitrate. The inhibitory effect of spermine was, therefore, studied at different intervals of induction in relation to NR activity, nitrate content of the tissue as well as the metabolic pool of nitrate. Spermine inhibited NR activity progressively with period of induction by about 60% at the end of 3 hr (Table 31). The nitrate content of the tissue increased with induction period and it was more in spermine treated than in the control group. Metabolic pool of nitrate was decreased by 50% in the spermine treated tissue.

Like spermine, tungstate a specific inhibitor of NR

Table 29 : Effect of spermine on the uptake of nitrate by excised cotyledons of radish seeds

	Pretreatment	Nitrate content in medium (µmoles)	
		Expt. I	Expt. II
Dark	-	8.2	97
	Spermine	8.2	97
Light	-	7.5	96
	Spermine	8.2	96

Growth conditions were same as in Table 27.

Cotyledons were excised and pretreated with spermine (1 mM) for 30 min and NR was induced with 10 µmoles of KNO_3 in Experiment I and 100 µmoles in Experiment II for 3 hr in dark or light.

Left over nitrate in the medium was estimated.

Table 30 : Effect of spermine on nitrate reductase activity when added in the assay system

Addition	Nitrate reductase activity (units/g fr tissue) in	
	Light	Dark
-	1.9	1.3
Spermine	1.8	1.2

Growth conditions were same as in Table 27.

Cotyledons were excised and NR was induced with KNO_3 (10 mM) in dark or light.

Concentration of spermine in the assay system was 1 mM.

Table 31 : Effect of spermine on metabolic pool and nitrate content of excised cotyledons of radish seeds

Period of induction (hr)	Pre-treatment	Nitrate reductase activity (units/g fr tissue)	Nitrate content (μmoles/g fr tissue)	Metabolic pool of nitrate (units/g fr tissue)
0	-	0.01	0.0	-
0.5	-	0.21	0.23	-
	Spermine	0.21	0.21	-
1.0	-	0.51	1.05	-
	Spermine	0.22	1.73	-
2.0	-	0.79	1.44	0.020
	Spermine	0.36	2.57	0.008
3.0	-	1.19	2.08	0.028
	Spermine	0.47	2.66	0.013

Seeds were germinated in dark for 36 hr in the absence of nitrogen source. Cotyledons were excised and pretreated with spermine (1 mM) for 30 min before induction with KNO_3 (10 mM) in dark for 3 hr. Nitrate content, NR activity and metabolic pool of nitrate were measured at specified periods of induction as given in materials and methods.

also caused a 94% inhibition and a higher nitrate content as compared to control (Table 32).

To determine whether spermine interferes with the induction of the enzyme, the effect of spermine and cycloheximide was compared when added at different intervals of induction (Tables 33 and 34). Both spermine and cycloheximide were more effective when added along with nitrate. Cycloheximide was more effective than spermine, causing a 90% inhibition, while spermine brought about 60% inhibition. The inhibitory effect of both the compounds decreased when added at later stages of induction. There was no effect when spermine was added at 2nd hr of induction, while cycloheximide caused only 30% inhibition. Thus, spermine like cycloheximide may inhibit the synthesis of the enzyme.

NR in addition to reducing nitrate is also known to have diaphorase activity (Beevers and Hageman, 1969). The effect of spermine was studied on the partial activities of NR, cytochrome c reductase and methyl viologen-NR. Spermine inhibited both the activities (Table 35)

Since the NR activity observed in the tissue at any given time is a net result of the balance between synthesis and degradation of the enzyme, the effect of spermine was

Table 32 : Effect of tungstate and spermine on the induction of nitrate reductase activity and nitrate content of excised cotyledons of radish seeds

Pretreatment	Nitrate reductase activity (units/g fr tissue)	Nitrate content (μmoles/g fr tissue)
-	1.10	2.15
Spermine	0.36	2.56
Na-tungstate	0.09	2.91

Growth and induction conditions were same as in Table 31.

Concentration of spermine used was 1 mM and Na-tungstate 10 mM.

Table 33 : Effect of spermine at different periods of induction of nitrate reductase activity in excised cotyledons of radish seeds

	Nitrate reductase activity (units/g fr tissue)
Control	0.93 (100)*
+ Spermine added during induction at (hr)	
0	0.37 (39)
0.5	0.41 (44)
1.0	0.81) (87)
2.0	0.95 (102)
2.5	0.98 (106)

Growth conditions were same as in Table 31. Cotyledons were excised and treated with spermine (1 mM) during induction as specified. Induction was carried out with KNO_3 (10 mM) for 3 hr.

* Figures in the parentheses represent per cent of control value taken as 100.

Table 34 : Effect of cycloheximide added at different periods of induction of nitrate reductase activity in excised cotyledons of radish seeds

	Nitrate reductase activity (units/g fr tissue)
Control	0.67 (100)*
+ Cycloheximide added during induction at (hr)	
0	0.007 (11)
0.5	0.15 (22)
1.0	0.27 (40)
2.0	0.47 (70)
2.5	0.68 (100)

Growth and induction conditions were same as in Table 33.

Concentration of cycloheximide used was 2 µg/ml.

* Figures in parentheses represent per cent of control value taken as 100.

Table 35 : Effect of spermine on partial activities of nitrate reductase

Treatment	Enzyme activity (units/g fr tissue)		
	NADH-nitrate reductase	Methyl viologen-nitrate reductase	Cytochrome c reductase
-	1.00	0.91	1.19
+ Spermine	0.40	0.38	0.51

Growth and induction conditions were same as in Table 31. Enzyme activities were assayed as given in materials and methods.

also studied on the stability of NR during storage, both *in vitro* and *in vivo*.

In vitro stability was studied either by storage of the homogenate of the tissue in which NR was induced in the absence or presence of spermine (Table 36) or by adding spermine to the homogenate during storage (Table 37). The rate of decay of the enzyme induced in the presence of spermine (Table 36). or when spermine was added to the homogenate after induction (Table 37) did not differ significantly from that of the control group either in light or dark.

However, spermine brought about a faster decrease in the level of NR in both light and dark when added during storage *in vivo* (Table 38). Light increased the activity of NR in control but not in the spermine treated group till the 2nd hr of storage. Also, there was about 40% decrease in NR activity of the control group when stored in the dark than in light where there was no loss of activity over a period of 10 hr when compared with 0 hr value. However, when compared with 2nd hr value there was about 20% decrease at 10 hr. In the spermine treated group however, there was a progressive decrease in NR level which reached to about 60% in 10 hr and this decrease was obtained in cotyledons stored in either light or dark.

Table 36 : Effect of spermine on the stability of nitrate reductase *in vitro*

Period of storage (hr)	Nitrate reductase activity (units/g fr tissue) in Light		Dark	
	-	+ spermine	-	+ spermine
0	1.23 (100)*	0.75 (100)	1.0 (100)	0.24 (100)
2	1.17 (95)	0.67 (89)	0.93 (93)	0.20 (83)
3	1.00 (81)	0.58 (78)	0.77 (77)	0.16 (67)
4	0.86 (70)	0.49 (68)	0.64 (64)	0.13 (54)
6	0.67 (54)	0.43 (44)	0.47 (47)	0.10 (42)

Seeds were germinated in dark for 36 hr in the absence of a nitrogen source. Cotyledons were excised and treated with spermine (1 mM) for 30 min before induction with KNO_3 (10 mM) in light or dark for 3 hr. Cotyledons were then homogenized and the homogenate was stored at 4° in dark. NR activity was assayed at specified periods of storage.

* Figures in parentheses represent per cent of zero hour value taken as 100.

Table 37 : Effect of spermine on the stability of nitrate reductase *in vitro*

Period of storage (hr)	Nitrate reductase activity (units/g fr tissue) in Light		Dark	
	-	+ spermine	-	+ spermine
0	1.26 (100)*	-	0.89 (100)*	-
2	1.13 (90)	1.12 (89)	0.80 (90)	0.81 (91)
3	1.01 (80)	1.01 (80)	0.67 (75)	0.65 (75)
4	0.90 (72)	0.92 (73)	0.55 (62)	0.53 (60)
6	0.76 (60)	0.76 (60)	0.45 (51)	0.44 (49)

Growth conditions were same as in Table 36.

Cotyledons were excised and NR induced in dark or light with KNO_3 (10 mM) for 3 hr. Cotyledons were then homogenized and the homogenate was stored at 4° in dark, in the absence or presence of spermine (1 mM). NR activity was assayed at specific periods of storage.

* Figures in parentheses represent per cent of zero hour value taken as 100.

Table 38 : Effect of spermine on the stability of nitrate reductase *in vivo*

Period of storage (hr)	Nitrate reductase activity (units/g fr tissue) in Light		in Dark	
	-	+ spermine	-	+ spermine
0	0.98 (100)*	-	0.98 (100)*	-
1	0.99 (101)	0.94 (96)	0.92 (94)	0.89 (91)
2	1.34 (137)	0.68 (69)	0.92 (94)	0.56 (57)
4	1.22 (124)	0.56 (57)	0.93 (95)	0.49 (50)
6	1.11 (113)	0.40 (41)	0.80 (82)	0.39 (40)
8	1.10 (112)	0.40 (41)	0.78 (80)	0.40 (40)
10	1.06 (108)	0.32 (37)	0.62 (63)	0.36 (37)

Seeds were germinated in dark for 36 hr in the absence of a nitrogen source. Cotyledons were excised and NR induced with KNO_3 (10 mM) in dark for 3 hr. Cotyledons were then washed with distilled water and stored in light or dark in the absence or presence of spermine (1 mM). NR activity was assayed at the specified periods of storage.

* Figures in parentheses represent per cent of zero hour value taken as 100.

The faster decay in NR activity in presence of spermine could have been due to an inhibitor of NR found in the cotyledons of seeds grown in the absence of a nitrogen source (Table 39). The presence of an inhibitor of NR has been reported earlier in radish cotyledons (Stulen et al., 1971). In the present study, the inhibitor was found to be specific for NR and did not inhibit either NiR or GS activity. Maximum inhibition obtained with the inhibitor was 60% and increasing the concentration further did not give any further inhibitory effect (Table 40). The inhibitor was found to be resistent to heat upto 60°, beyond which it was gradually inactivated and at 100° it was totally inactive (Table 41). The inhibitor was found in the supernant fraction (Table 42) and was dialyzable (Table 43). Spermine however, did not have any effect on the inhibitor (Table 44). Pretreatment of the inhibitor with NADH brought about an increased inhibition of NR activity (Table 45) whereas pretreatment with $K_3Fe(CN)_6$ abolished the effect of the inhibitor. Addition of PVP to the extraction medium of NR did not enhance the NR activity, on the other hand, it inhibited NR (Table 46). When the level of protease was studied during the induction and storage of NR, spermine was found to increase the level of protease by about 30% (Table 47). This was similar to the effect observed during germination.

Table 39 : Effect of inhibitor on nitrate reductase, nitrite reductase and glutamine synthetase activity when added in the assay system

	Nitrate reductase	Nitrite reductase	Glutamine synthetase
	(units/g fr tissue)		
-	0.93 (100)*	6.0 (100)	11.0 (100)
+ Inhibitor	0.40 (43)	7.1 (118)	11.5 (101)

Seeds were grown in dark in the absence of a nitrogen source for 36 hr. Cotyledons were excised and treated with KNO_3 (10 mM) for 3 hr. These cotyledons were used as source of enzymes.

For inhibitor, uninduced cotyledons were homogenized in the grinding medium used for NR (10% w/v). 0.2 ml of this homogenate was added to the assay system (containing NR 0.02, NiR 1.8 and GS 1.8 units/g fr tissue).

* Figures in the parentheses represent per cent of control value taken as 100.

Table 40 : Effect of inhibitor concentration on nitrate reductase activity

Concentration of inhibitor (ml)	Nitrate reductase activity (units/g fr tissue)
0	1.00 (100)*
0.1	0.74 (74)
0.2	0.42 (42)
0.4	0.40 (40)
0.5	0.40 (40)

Growth, induction and assay conditions of NR activity was same as in Table 39.

* Figures in the parentheses represent per cent of control value taken as 100.

Table 41 : Effect of temperature on inhibitor of nitrate reductase from radish cotyledons

Treatment	Nitrate reductase activity (units/g fr tissue)
-	0.90 (100)*
+ Inhibitor unheated	0.34 (38)
+ Inhibitor heated at °	
40	0.39 (43)
60	0.38 (43)
80	0.47 (53)
90	0.77 (85)
100	0.95 (106)

Growth conditions and induction of NR were same as in Table 39.

The inhibitor extract was heated for 10 min at the specified temperature and then added to the assay system.

* Figures in the parentheses represent per cent of control value taken as 100.

Table 42 : Localization of inhibitor of nitrate reductase in radish cotyledons

Treatment	Nitrate reductase activity (units/g fr tissue)
-	0.89 (100)*
+ Inhibitor	
Homogenate	0.32 (36)
Supernant	0.36 (41)
Residue	0.84 (95)

Growth conditions and induction of NR activity was same as in Table 39. The inhibitor homogenate was centrifuged at 40,000 x g for 20 min.

* Figures in the parentheses represent per cent of the control value taken as 100.

Table 43 : Effect of dialysis on the inhibitor of nitrate reductase from radish cotyledons.

Treatment	Nitrate reductase activity (units/g fr tissue)
-	1.10 (100)*
+ Inhibitor (undialyzed)	0.45 (44)
Dialyzed	1.25 (112)

Growth conditions and induction of NR activity was same as in Table 39. The inhibitor homogenate was dialyzed against the grinding medium for 6 hr.

* Figures in the parentheses represent per cent of the control value taken as 100.

Table 44 : Effect of spermine and inhibitor on nitrate reductase activity in cotyledons of germinating radish seeds

Treatment	Nitrate reductase activity (units/g fr tissue)	
	Dark	Light
-	0.92 (100)*	-
+ Inhibitor from cotyledons without treatment with spermine	0.37 (40)	0.43 (47)
+ Inhibitor from cotyledons treated with spermine	0.39 (42)	0.41 (45)

Growth conditions and induction of NR activity was same as in Table 39.

For inhibitor study, cotyledons were stored for 3 hr in the absence or presence of spermine (1 mM), homogenized and the inhibitory effect tested.

* Figures in the parentheses represent per cent of the control value taken as 100.

Table 45 : Effect of NADH and $K_3Fe(CN)_6$ on the inhibitor of nitrate reductase in cotyledons of germinating radish seeds

	Nitrate reductase activity (units/g fr tissue)
-	0.80 (100)*
+ Inhibitor Pre-treated with	
-	0.32 (40)
NADH	0.23 (28)
$K_3Fe(CN)_6$	1.02 (128)

Growth and induction of NR was same as in Table 39. The inhibitor homogenate was pretreated with either NADH (0.1 mM) or $K_3Fe(CN)_6$ (0.3 mM) for 15 min and then added to the assay system.

* Figures in the parentheses represent per cent of the control value taken as 100.

Table 46 : Effect of PVP on nitrate reductase activity in cotyledons of germinating radish seedlings

Treatment	Nitrate reductase activity (units/g fr tissue)
-	1.01 (100)*
+ PVP 1:1	0.86 (85)
1:2	0.44 (44)
1:3	0.39 (39)

Growth and induction of NR activity were same as in Table 39. Extraction of NR activity was carried out in the presence of PVP in increasing proportions to the fresh weight of the cotyledons.

* Figures in the parentheses represent per cent of the control value taken as 100.

Table 47 : Effect of spermine on protease activity during induction and storage of excised radish cotyledons

Period (hr)	Compound	Nitrate reductase (units/g fr tissue)	Protease
Induction			
0	-	0.09	6.2
3	-	1.09	6.3
	+ spermine	0.40	8.4
Storage			
3	-	1.00	6.9
	+ spermine	0.25	8.9

Seeds were germinated in dark for 36 hr in the absence of a nitrogen source. Cotyledons were excised and treated with spermine (1 mM) for 30 min before induction with KNO_3 (10 mM) in dark for 3 hr. Cotyledons were then washed and stored in dark. NR and protease activity were assayed at specified period of induction and storage.

During germination of seeds, GS was found to be inhibited by the compounds in light grown seeds in presence of nitrate (Table 11) while AAT was increased in light grown seeds irrespective of the presence of nitrogen sources (Tables 19,20,21). Since light increases chloroplastic GS (Nishimura et al., 1982) as well as AAT activity (Kirk and Leech, 1972) but not the cytoplasmic enzymes, the effect of spermine was studied in the chloroplastic fraction. The results reported in Table 48 show that when the seeds were germinated in the absence or presence of spermine, transferred to light for 24 hr and subcellular fractionation was carried out, spermine inhibited only the GS activity of chloroplastic fraction while that of the supernant was not affected. Similarly, AAT of the chloroplastic fraction only was activated and not that of the supernant fraction.

The increase of protease (Tables 16,17,18) and the chloroplastic AAT (Table 48) by the compounds could be either due to the activation of preexisting enzyme or due to de novo synthesis. The results reported in Table 49 show that the increase in protease by spermine was not affected by cycloheximide while that of AAT was abolished by treatment with cycloheximide suggesting that the increase in protease activity was due to activation while that of AAT was due to increased synthesis.

Table 48 : Effect of spermine on glutamine synthetase and alanine aminotransferase in subcellular fractions of radish cotyledons

Treatment	Glutamine synthetase (units) in		Alanine aminotransferase (units) in	
	Chloroplast	Supernatant	Chloroplast	Supernatant
Seeds grown in dark for 72 hr				
Control	4	42	1	12
+ Spermine*	3	42	1	12
Seeds grown in dark for 48 hr and then transferred to light for 24 hr				
Control	21	42	5	12
+ Spermine	11	39	10	12

* Spermine concentration was 1 mM.

Table 49 : Effect of spermine, GAA and cycloheximide on protease and alanine aminotransferase activity in the cotyledons of radish seeds

Treatment	Protease (units)	Alanine aminotransferase (units)
-	7.4	21
Spermine*	9.8	33
GAA*	10.0	32
Cycloheximide*	7.2	15
Spermine + Cycloheximide	9.5	17
GAA + Cycloheximide	9.6	18

* The concentration of spermine and GAA were 1 mM while that of cycloheximide was 0.5 µg/ml.