for 20 min, the E_{750} was determined in a spectrophotometer (1 cm light-path cell). A standard series for P_i (0.04 µmoles of P_i) was determined each time. Protein was determined by the method of Lowry et al.[14] using bovine serum albumin as standard.

Results and discussions. ATP-sulphurylase was found in the supernatant fractions (S_{13}, S_{144}) (table). Cell-free preparations from 10-day-old cultures showed the maximum enzyme activity. (Figure 1 shows that maximum activity was obtained when the cell paste was disrupted by ultrasonicator (20 Kcys/sec) for 90 sec. The optimum pH of the enzyme was between 7 and 8 (figure 2). From 30 to 40 °C, there was a rapid increase in the enzyme activity, after which it declined (figure 3). Boiled enzyme (2 min) was completely inactive. The enzyme activity was stable for over 21 days when kept at − 6 °C.

The localization of ATP-sulphurylase in soluble subcellular fractions of cells, activity over the broad pH range of 7–8, are in general agreement with earlier studies[8] on enzyme from *Anabaena cylindrica.* Contrary to a report on *A. cylindrica*[8], the enzyme fraction from *S. platensis* is labile at temperatures higher tahn 50 °C.

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Effect of phytic acid on diamine oxidase activity in germinating pea seeds

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Summary. Diamine oxidase present in the cotyledons of germinating pea seeds is induced by phytic acid but the embryo enzyme is not affected. Polyamines have no effect on phytase activity of the cotyledon or embryo.

Diamine oxidase (E.C.1.4.3.6) of plants, which oxidizes several di- and polyamines as well as primary amines[3–5], has been extensively characterized from pea seedlings[3–6]. Studies from this laboratory have shown[7] that the pea enzyme, which may have a role in the synthesis of indole-3-acetic acid, is regulated in a feed-back manner by auxins and is induced by its substrates. On feeding 2,4-D to intact pea seedlings, the activity of the cotyledon diamine oxidase is reduced[7]. This effect is mediated through the embryo since its removal after soaking the seeds in 2,4-D for 14 h abolished the inhibitory effect, suggesting the elaboration of an inhibitor of diamine oxidase activity or synthesis in the embryo. The inhibitory effect of 2,4-D on the enzyme activity in the cotyledon, which may be mediated through ethylene, is reversed by exposure of seeds to red light[8].

Polyamines being cationic compounds are known to form complexes with polyanions such as nucleic acids[9,10]. It seemed worth investigating whether phytic acid, another polyanionic compound, has any control on the polyamine metabolism. The results of this study are reported in the present communication.

Materials and methods. Pea seeds *(Pisum sativum)* were surface sterilized with lysol and throughly washed with water and then soaked for 14 h in distilled water or other test material as specified. The seeds were then kept for germination at 22 °C in the dark in Petri dishes on moist filter papers. In cases where the embryo was removed before or after soaking, the cotyledons were kept in Petri dishes on moist filter papers for the same period of time. The time of the commencement of treatment or soaking of the seeds was considered as zero time. At specified periods cotyledon and embryo were separated and a 10% extract of the tissue was prepared by grinding with a pestle in a chilled mortar using 60 mM phosphate buffer, pH 7. The extract after passing through 2 layers of cheese cloth was used for enzyme assays. Diamine oxidase activity was determined as described earlier[7]. Phytase activity was determined according to the method of Mandal et al.[11]. 1 unit of diamine oxidase has been defined as the amount of enzyme required to form 1 µmole of Δ'-pyrroline/min under the assay conditions.

Results and discussion. In whole seeds where the emryo was present during soaking and germination, the cotyledon diamine oxidase was induced by phytic acid (table 1). The induction of cotyledon enzyme was not affected by the removal of the embryo after soaking but its removal before soaking induced the cotyledon enzyme to a lesser extent, when compared with that obtained in cotyledons from

Table 1. Effect of phytic acid on pea cotyledon and embryo diamine oxidase activity

Concentration of phytic acid (ppm)	Enzyme units/g fresh tissue in Cotyledon						Embryo	
	Seeds soaked and germinated with embryo		Embryo removed from seeds before soaking		Embryo removed from seeds after soaking			
	38 h	62 h	38 h	62 h	38 h	62 h	38 h	62 h
0	0.02	0.45	0	0	0.07	0.48	0.25	0.69
200	0.28	0.65	0	0.07	0.25	0.66	0.28	0.71
400	0.32	0.73	0.04	0.16	0.38	0.78	0.30	0.68

Values are means from duplicate samples.

whole seeds or where the embryo was removed after soaking. The embryo enzyme, however, was not affected by phytic acid. A dose response study showed (table 2) that a minimum of 15–20 ppm of phytic acid is needed for induction of the cotyledon enzyme. After 62 h of germination, however, there was no difference in control or phytic acid treated groups.

Phytic acid has been shown to accumulate in seeds[12] and during germination it decreases as a result of increased phytase (E.C.3.1.3.8) activity to provide the phosphate required for growing tissue[13]. Phytic acid, being a polyanion like nucleic acids, may also form complexes with polyamines. The induction of cotyledon diamine oxidase by phytic acid suggests that the levels of polyamines may be controlled by phytic acid. To investigate whether polyamines have any effect on phytase activity, to maintain a balanced concentration of phytic acid and polyamines, studies were carried out by soaking the seeds in 0.1% putrescine, cadaverine, spermidine and spermine. These compounds had no effect on the phytase activity of the cotyledon or embryo, suggesting that polyamines may not have any control over phytic acid levels. The inducing effect of phytic acid was not due to a higher level of phosphate or inositol since these compounds could not induce the cotyledon enzyme when the seeds were soaked in their presence.

Table 2. Effect of phytic acid on pea cotyledon and embryo diamine oxidase activity

Concentration of phytic acid (ppm)	Enzyme units/g fresh tissue in Cotyledon				Embryo	
	Seeds soaked and germinated with embryo		Embryo removed from seeds after soaking			
	38 h	62 h	38 h	62 h	38 h	62 h
0	0.02	0.43	0.07	0.53	0.25	0.69
5	0.05	0.44	0.08	0.51	0.29	0.66
10	0.09	0.47	0.09	0.56	0.31	0.72
15	0.11	0.49	0.09	0.61	0.29	0.74
20	0.20	0.64	0.21	0.66	0.28	0.77
25	0.25	0.68	0.22	0.76	0.33	0.82

Values are means from duplicate samples.

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Lack of effect of neurotransmitters on cyclic AMP phosphodiesterase activity in an insect CNS[1]

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Summary. The inhibition of cyclic nucleotide phosphodiesterase from the nerve cord of *Manduca sexta* was studied using theophylline as a model compound. 11 putative neurotransmitters had no effect on enzyme activity.

In previous work by our laboratory we have studied the action of putative neurotransmitters on the accumulation of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in intact nerve cords[4] of the tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae), in neuronal- and glial-enriched cellular fractions derived from it[5], and on the activity of adenylate cyclase (E.C. 4.6.1.1) in crude homogenates of this tissue[6]. The activity of the cyclic nucleotide phosphodiesterase (PDE, E.C. 3.1.4.1c) in this tissue was thoroughly explored enzymatically in this laboratory[7], but a detailed pharmacological investigation was required to support the hypothesis that neurotransmitters effect elevation of cyclic AMP levels by stimulation of the adenylate cyclase rather than by inhibition of the PDE[4,6,8]. The results of this study have been presented in abstract form[9].

Materials and methods. Nerve cords from 5th instar 'wanderer' larvae[10] were dissected and frozen as previously described[7]. Freshly-thawed nerve cords were homogenized in all-glass tissue grinders in 50 mM Hepes-KOH, pH 7.5, 152 mM NaCl, 4.7 mM KCl, 2.8 mM $CaCl_2$[11], and centrifuged at 34,000 × g for 30 min to remove insoluble debris.

PDE activity was quantitated essentially by assay II[7]. Brie-

Effects of putative neurotransmitters on cyclic AMP phosphodiesterase activity

Addition	Phosphodiesterase activity (pmole cyclic AMP/ µg protein/min)
None	1.83 ± 0.06 (21)
Acetylcholine 50 mM	1.95 ± 0.14 (3)
Serotonin 10 mM	1.52 ± 0.21 (3)
Aspartic acid 500 µM	1.88 ± 0.09 (3)
Glutamic acid 500 µM	1.74 ± 0.16 (5)
Glycine 500 µM	1.97 ± 0.10 (5)
Gamma-aminobutyric acid 500 µM	1.70 ± 0.09 (6)
Epinephrine 250 µM	1.76 ± 0.05 (3)
Norepinephrine 250 µM	1.75 ± 0.07 (3)
Isoproterenol 250 µM	1.62 ± 0.06 (3)
Octopamine 250 µM	1.62 ± 0.03 (5)
Dopamine 250 µM	0.84 ± 0.06 (14)*
Phenylthiourea 20 µM	1.58 ± 0.16 (5)
Phenylthiourea 20 µM + Dopamine 250 µM	1.47 ± 0.14 (5)

Results are given as mean ± SE. The number of samples is given in parentheses. Statistical significance was determined using Student's t-test. * $p < 0.001$ vs no addition.