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EFFECT OF TEMPERATURE OF IMBIBITION ON PHOSPHOLIPID METABOLISM IN PEA EMBRYONIC AXES

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Key Word Index—*Pisum sativum*; Leguminoseae; seeds; germination; phospholipids; phosphatidyl choline; temperature; endoplasmic reticulum; plasma membrane; choline incorporation.

Abstract—Pea seeds were imbibed in radioactive choline and then germinated. Treatments were either at 5° or 25° and the seeds were imbibed for 5 hr at one temperature and then transferred to the other. [Me-^{14}C]Choline incorporation into phosphatidyl choline in the ER and the plasma membrane obtained from the embryonic axes after germination was measured. Seeds kept constantly at 25° had a very rapid initial incorporation of choline followed by a loss of label. Seeds kept at 5° had a very much lower rate of incorporation. However, seeds transferred from 5 to 25° behaved for at least 48 hr as if continuously kept at 5°, while in seeds transferred from 25 to 5° incorporation stopped after 15 hr. The seeds apparently respond to transient exposure to temperature by a changed metabolism of phospholipid. Data are also given for the choline content of the seeds under the different treatments and for the changes in total phospholipid.

INTRODUCTION

Seeds respond to the temperature in their environment and their germination behaviour is determined by the ambient temperature. Not only do seeds germinate in some definite temperature range but they also respond by delaying germination after exposure for relatively brief periods to temperatures unfavourable to germination. We have suggested that the mechanism by which seeds sense temperature is associated with cellular membranes [1]. The extensive literature supporting this view was reviewed by Mayer [1] and by Mayer and Marbach [2]. The metabolism of membranes in seeds has been studied to some extent [3–8]. Attention has been paid especially to metabolism of membranes in the aleurone of barley and wheat [9, 10]. However, relatively little attention has been paid to the effect of temperature on the metabolism of seed membranes. Since the pathways of phospholipid metabolism in plants are fairly well known [11, 12] and since they are metabolised with great rapidity we decided to study the response of phospholipid metabolism to temperature in germinating pea seeds. The present paper reports some of the results.

RESULTS AND DISCUSSION

In the experiments to be described here we adopted the experimental approach previously described [6] by which pea (*Pisum sativum* L. cv Alaska) seeds were fed radioactive precursors during imbibition and the fate of the precursor followed during germination. Since we intended to study the effect of temperature, its effect on imbibition of water was first determined (Fig. 1). As can be seen, the initial effect of temperature on imbibition was appreciable, but at 5° after 5 hr imbibition was 80% of that at 25°. This allowed us to apply the technique previously described. Seeds were imbibed at different temperatures for 5 hr at 25° or 6 hr at 5° in the presence of [Me-^{14}C]choline, and then germinated for various periods of time. The embryonic axes were removed after the suitable period of time, membrane fractions isolated and phospholipid extracted. The increase in fresh weight of the embryos, which is a measure of their growth, is shown in Fig. 2. Treatment at 5°, either continuously or after imbibition at 25°, practically stopped growth. However, the transfer from 5 to 25° caused a lag in the increase of fresh weight, which amounted to less than 25% after 15 hr. The incorporation of radioactive choline into phosphatidyl choline was then determined. The results are shown in Figs 3 and 4 expressed as incorporation per g dry wt or fr. wt of embryonic axis. Two temperatures were selected for study: 25° and 5°. Seeds were either imbibed and germinated at the same temperature 25° to 5°. The results were quite clear cut. The axes of seeds maintained at 25° showed a rapid incorporation of choline into both the ER and the plasma membrane. The most striking change observed was in the ER. However, as germination proceeded label was again lost, either due to turnover or breakdown. The axes of seeds maintained at 5° showed very little incorporation into either membrane fraction, as might be expected from the low rate of metabolism, at 5°. Incorporation after 48 hr at 5° was less than 10% of the peak value at 25° in the ER and about 40% in the plasma membrane. Seeds imbibed at 5° and then transferred at 25° showed a lag of choline incorporation, the rate being similar to that of seeds maintained all the time at 5°, both in the ER and in the plasma membrane. In seeds which were transferred from 25° to 5° there was initial incorporation into the ER which ceased after some 15 hr, while incorporation into plasma membrane was delayed for 24 hr and then slowly rose. It appears as if an imbibition temperature of 5° determines subsequent behaviour of choline metabolism for a further 48 hr, but imbibition at 25° did not have the same effect. These results could be due to a number of possible causes. It was possible that the seeds lost appreciable amounts of label after imbibition at

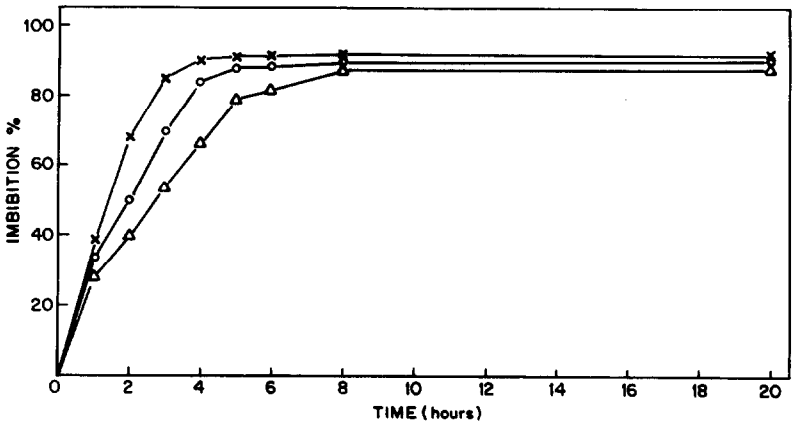

Fig. 1. Effect of temperature on imbibition of water by peeled pea seeds. △—△, 5°; ○—○, 15°; ×—×, 25°. (S.E. was less than 1%.)

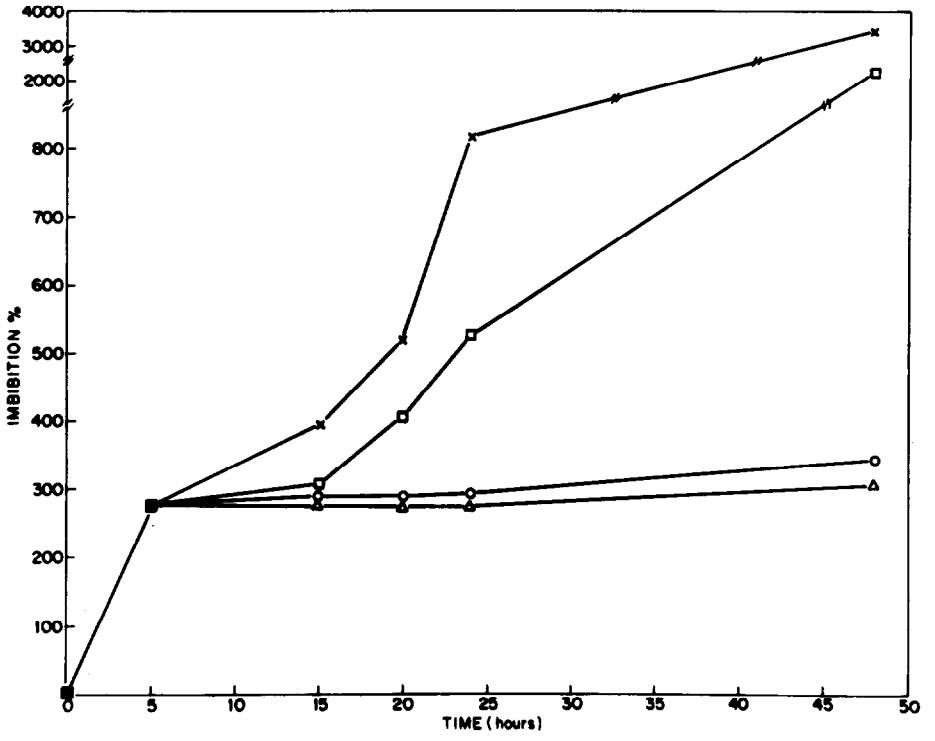

Fig. 2. Effect of temperature on the increase in fresh weight of the embryonic axes of peeled pea seeds. △—△, imbibition and germination at 5°; ×—×, imbibition and germination at 25°; □—□, imbibition at 5° and germination at 25°; ○—○, imbibition at 25° and germination at 5°. (S.E. was less than 1%.)

5° followed by transfer to 25°. However, we found that seeds contained 70% of the applied label after 24 hr of germination, the bulk of which, 97.5%, was present in the cotyledons and 2.5% in the embryonic axes, consistent with the ratios of dry wts of cotyledon to embryonic axes. The total loss from the seeds after 24 hr of germination was 3.6% of uptake, under conditions of transfer from 5 to 25°. This could not therefore account for the failure of the embryos to incorporate label into membranes.

A second possibility was that the endogenous pool of choline in the embryonic axis changed markedly after imbibition at 5°. We therefore measured endogenous choline with length of germination period (Fig. 5). The endogenous pool of choline, expressed as per g fr. wt dropped quite markedly in the seeds maintained at 25°. In seeds transferred from 5 to 25° there was an initial increase followed by a drop after 15 hr. The difference in pool size after 15 hr was 75%. After 20 hr the difference was only 68%. It seems very unlikely that this difference is responsible for the very large difference in incorporation of choline into phosphatidyl-choline. It is interesting to note that treatment at 5° induces an increase in choline content.

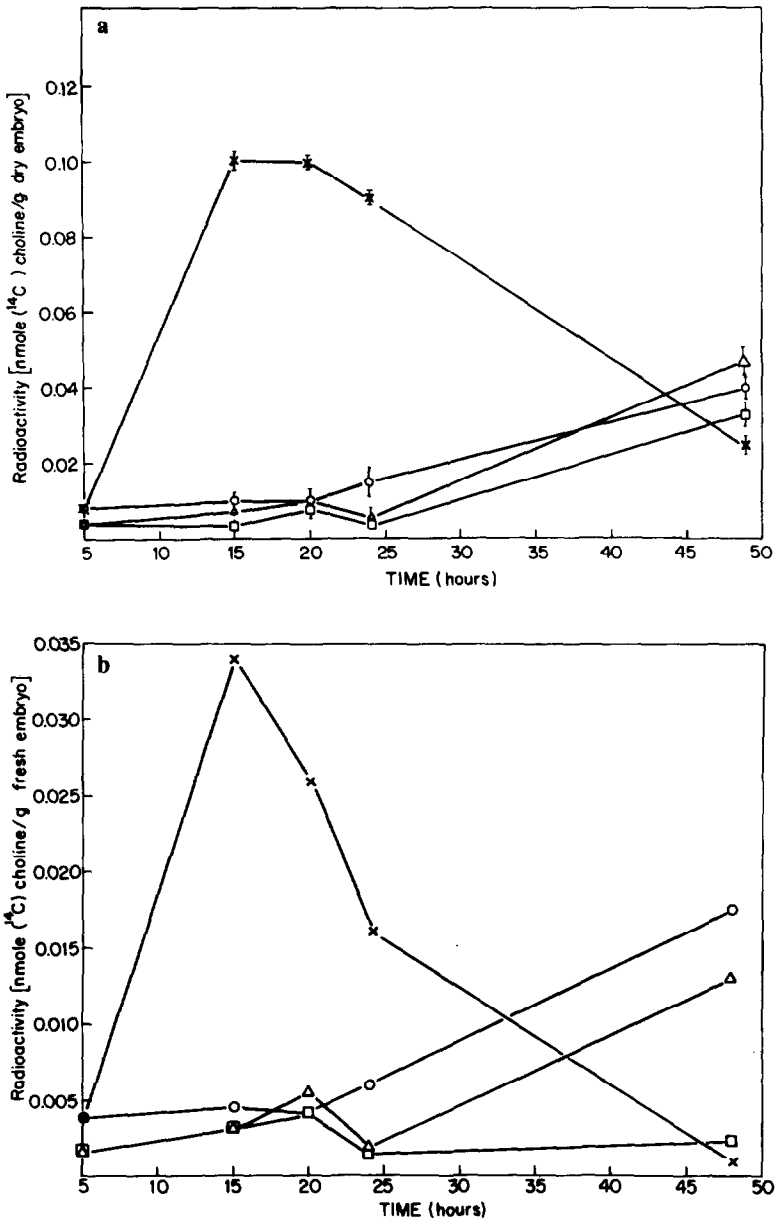

Fig. 3. Effect of temperature on incorporation of choline into phosphatidyl choline in plasma membrane of germinating pea embryonic axis. A, Results/g dry embryo; B, results/g fresh embryo. (Result ± S.D.). Symbols as in Fig. 2.

Even transfer from 25 to 5° resulted in a subsequent increase in the choline content.

Lastly, it was possible that the temperature response was due to changes in the total amount of phospholipid in the seeds. We therefore measured changes in total phospholipid content of the ER and the plasma membrane fraction in the embryonic axes (Table 1). In the embryonic axes initial lower temperature depressed synthesis of phospholipid in the plasma membrane during the first 5 hr. During this period phospholipid content of the ER was not affected. After 20 hr the phospholipid content per g fr. wt was similar in all treatments for plasma membrane, but dropped in the ER in seeds maintained at 25°, presumably due to water uptake by the cells. This drop is not evident if the results are expressed on a dry wt basis. Only in seeds transferred from 5 to 25° did the ER phospholipid markedly change. The changes after 48 hr clearly reflect changes in growth and water content of the cells as indicated by the drop in phospholipid/fr. wt in seeds kept at 25° throughout or transferred from 5 to 25°. None of these changes can cause the change in choline incorporation into the phospholipid fraction. Indeed, it is debatable whether calculation of phospholipid content on a fr. wt basis is legitimate in a tissue taking up water so rapidly.

The results described here point to a new and un-

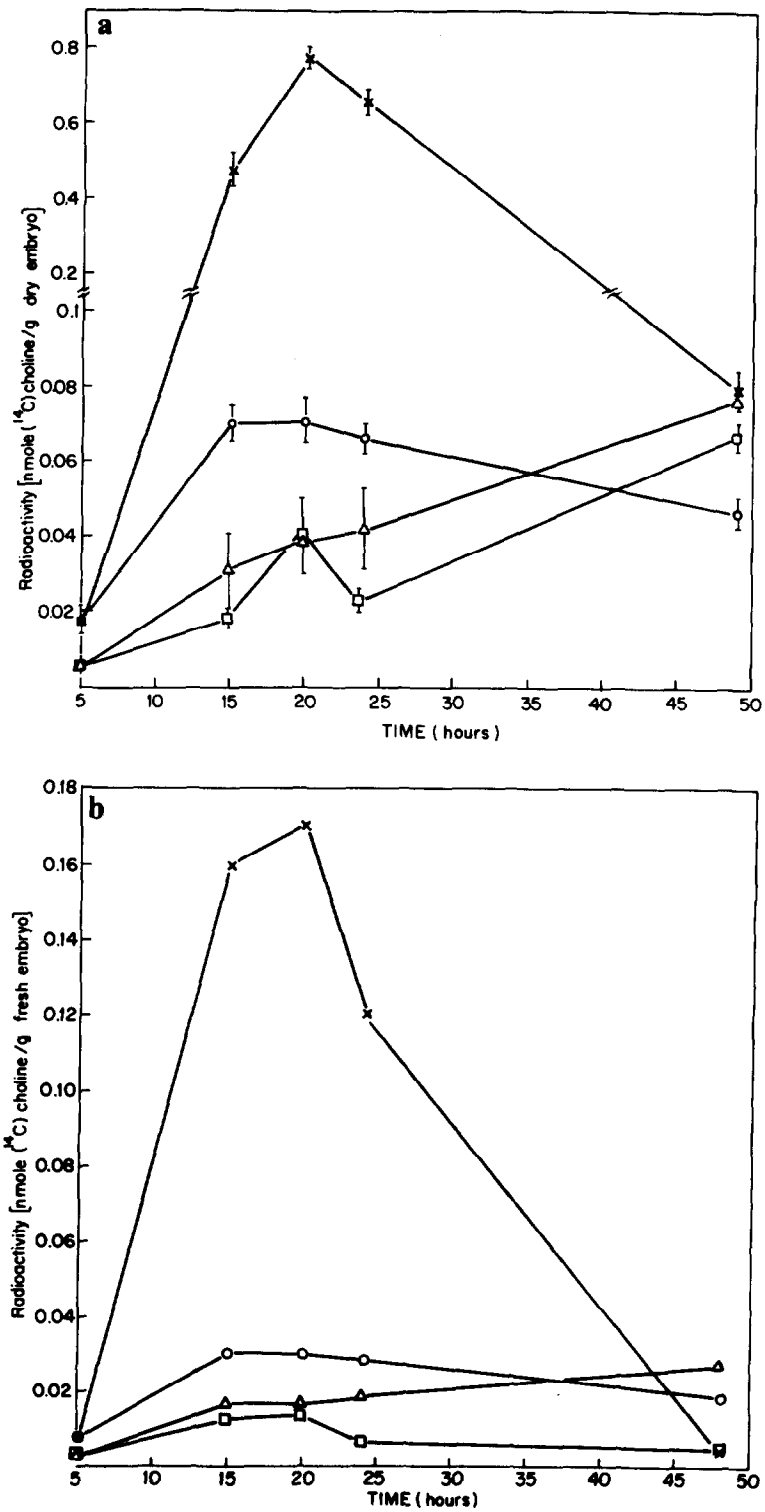

Fig. 4. Effect of temperature on incorporation of choline into phosphatidyl choline in endoplasmic reticulum of embryonic axis of germinating peas. A, Results/g dry embryo; B, results/g fresh embryo. Symbols as in Fig. 2.

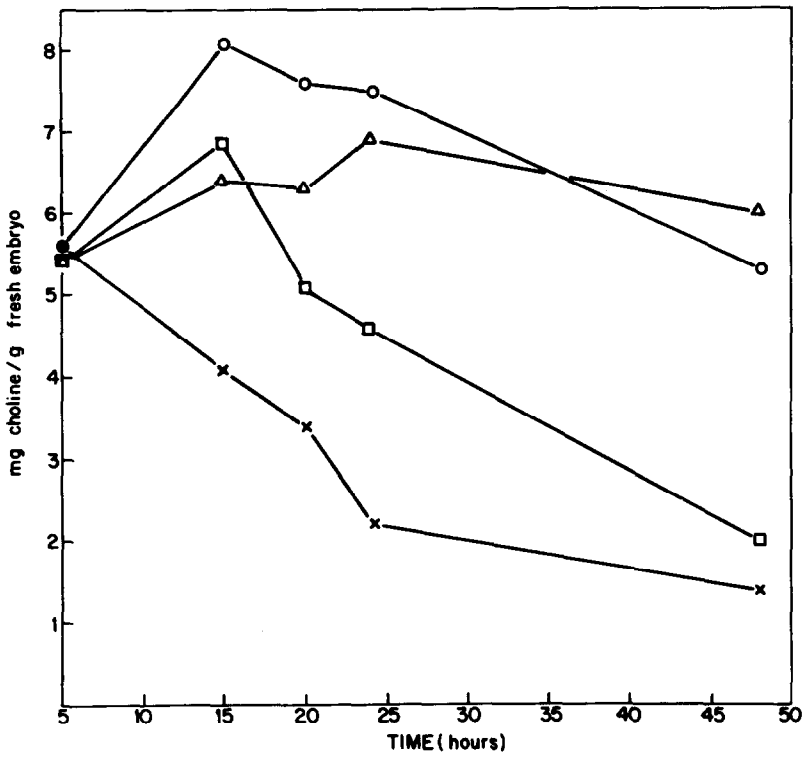

Fig. 5. Effect of temperature on the endogenous free choline content in embryonic axis of germinating peas. Symbols as in Fig. 2.

Table 1. Changes in phospholipid content of plasma membrane and endoplasmic reticulum during germination of embryonic axes of peas

Length of germination (hr)	Temperature treatment	Plasma membrane		Endoplasmic reticulum	
		(μmol P/g fr. wt)	(μmol P/g dry wt)	(μmol P/g fr. wt)	(μmol P/g dry wt)
5	25°→25°	1.6	3.5	1.82	4.05
	25°→5°	1.6	3.5	1.82	4.05
	5°→5°	0.66	1.5	1.8	4.05
	5°→5°	0.66	1.5	1.8	4.05
24	25°→25°	1.29	7.1	1.18	6.5
	25°→5°	1.42	3.3	2.6	6.0
	5°→5°	1.2	2.4	3.7	7.6
	5°→25°	1.73	6.5	2.9	10.9
48	25°→25°	0.24	5.1	0.72	15.2
	25°→5°	1.49	3.7	2.8	7.07
	5°→5°	2.4	6.1	2.8	6.9
	5°→25°	0.65	9.0	0.87	12.0

expected effect of temperature on seeds during germination. Exposure of pea seeds to a low temperature during imbibition, which delays but does not prevent germination, profoundly alters incorporation of choline into phosphatidyl choline in the embryonic axis, despite the fact that total phospholipid content increases if calculated on a dry wt. basis. The reverse transfer from 25 to 5° effectively reduces choline incorporation. Thus, while the exposure to the low temperature during imbibition determines subsequent choline metabolism, pretreatment with the favourable temperature does not result in a continuation of behaviour as if the seeds were at the higher temperature. Since brief exposure to low temperature might be supposed to prevent germination while brief

exposure to favourable temperature should not immediately trigger off full germination, the behaviour does show some consistency. We cannot at present suggest a mechanism which underlies these effects. It will be necessary to investigate the metabolism of other parts of the phospholipid, e.g. the glycerol backbone. Preliminary results indicate that incorporation of glycerol is also markedly affected by the transfer from 5 to 25° as compared with continuous exposure to 25°. The results will be reported in detail in due course. In addition we will have to determine whether enzymes responsible for synthesis of phosphatidyl cholines or their breakdown will respond to temperature treatment. Such experiments are now under way.

EXPERIMENTAL

Pea seeds cv. Alaska were obtained from the Ferry Morse Seed Co. The seeds were peeled prior to imbibition. Imbibition, germination and isolation of membranes, endoplasmic reticulum and plasma membrane were all done as previously described [6]. The seeds or seed parts were ground in Tris–MES buffer, 25 mM each, pH 7.5 containing 3 mM EDTA and 0.2 M sucrose. After removal of a particulate fraction by centrifugation at 13 000 *g* (15 min), the supernatant was centrifuged at 80 000 *g* for 30 min. The pellet was applied to a non-linear sucrose gradient [6] and centrifuged for 2 hr at 82 000 *g*. Two bands were removed, one at the interphase 1.17–1.19 density and the other at density 1.11–1.13, these fractions being termed 'plasma membrane' and 'endoplasmic reticulum', respectively. The isolated bands were collected, diluted and centrifuged down and used for subsequent analyses.

For determination of choline incorporation, phospholipids were extracted from the pellets according to [6] and [13]. Where appropriate, the phospholipids were separated by TLC [6, 14] and the zones located [13]. The zones were scraped off the TLC plates and radioactivity determined by scintillation counting. Radioactive [Me-^{14}C]choline chloride (58 mCi/mmole) was always supplied during imbibition, 5 μCi in 7 ml soln/2–3 g dry seed, and the seeds then rinsed and transferred to conditions for germination on moist cotton wool. When embryonic axes were examined, they were excised at the end of the experimental period. Total phospholipid in the membrane fractions was determined by isolating the fractions reprecipitating them after dilution by centrifugation at 80 000 *g* for 30 min, extracting total phospholipid and then measuring phosphate content as described by Fiske and Subba-Row [15]. Choline was measured using the Reinecke salt [16] as described in ref. [6].

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