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CARNITINE CONTENT OF PEA SEEDLING COTYLEDONS

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Key Word Index—*Pisum sativum*; Leguminosae; pea cotyledons; carnitine determination.

Abstract—Using a radioactive assay for the determination of carnitine, the amount in pea cotyledons was shown to vary with age.

INTRODUCTION

Carnitine has been detected in a variety of mammalian tissues (see review by Fritz [1]) and its presence in plants is known [2]. Carnitine has been detected in tissue extracts using a spectrophotometric assay based on the reaction

L-carnitine + acetyl − CoA →

acetyl-L-carnitine + CoA SH

which is catalyzed by the enzyme carnitine acetyl transferase (EC 2.3.1.7.). Reduced coenzyme A formed in this reaction has been estimated by the reaction of the –SH group of reduced CoA with 5,5[1]-dithiobis-2-nitrobenzoic acid or else by enzymic methods [3,4]. However, it is possible that extracts of tissue contain SH-groups which interfere with the determination of reduced CoA. A method first described by Cederblad and Lindstedt [5], in which the acetyl-L-carnitine formed in the reaction was measured, was therefore adapted to determine the L-carnitine content of germinating peas.

RESULTS AND DISCUSSION

Preliminary experiments established the concentration of carnitine-*O*-acetyltransferase to be used in assays for L-carnitine and also the optimum time of incubation. A calibration curve was prepared using known concentrations of carnitine in the incubation mixtures. This curve was linear for concentrations of L-carnitine from 0·25 to 2 nmol. The carnitine content of dry pea seeds (0 hr germination) was low in comparison to the content in cotyledons of germinated peas (Table 1) In the 24 hr following the soaking of peas in water, there was an increase in the carnitine content. This might be due to an increased synthesis of carnitine following imbibition or it is possible that carnitine might be released from a bound form during this period. In 48 hr and 72 hr pea cotyledons the carnitine content decreased. Thus, if carnitine is involved in transfer of fatty acyl-groups across, for example, mitochondrial membranes, as has been suggested for various mammalian mitochondria, then it might appear that such an involvement is at its maximum when stored fat reserves in the pea cotyledon are being utilized in the initial stages of germination.

Table 1. Carnitine content of germinating pea cotyledons

hr germinated	Carnitine content nmol per g fr wt	nmol per g dry wt
0	1·0	2·1
24	6·0	14·0
48	3·9	9·4
72	3·6	9·1

EXPERIMENTAL

Acetyl-coenzyme A (Na salt) and carnitine acetyl transferase was purchased from Sigma. Acetyl-(1-^{14}C)-coenzyme A and L-carnitine were purchased commercially.

Pea seeds (*Pisum sativum* var. Alaska) were soaked under running H_2O for 12 hr and germinated on moist blotting

paper in the dark at 25°. The period of germination was measured from the commencement of soaking.

Preparation of Extract. About 10 g fr wt of cotyledons from which the testas and radicles had been removed were ground in a pestle and mortar with 50 ml H_2O and a little acid-washed sand. In the preparation of extract from ungerminated peas the dry seeds were first ground in a mill before subsequent extraction as before in a mortar. 50 ml 1 M $HClO_4$ was then added to the mixture, which was ground further. Finally the slurry was made 50% with respect to EtOH and neutralized with KOH. The suspension was stood at 0° for 1 hr and the ppt removed by filtration. The filtrate was evaporated to dryness at 35° and the residue dissolved in H_2O to give a final vol. of 4 ml. To express the results on both fr. wt and dry wt basis, 10 batches of seeds (5 g each) were imbibed and germinated for the appropriate period. Values for 0 hr pea seeds were obtained by imbibing air-dry seeds in 1% $CuSO_4$ for 24 hr. The $CuSO_4$ prevents germination but allows imbibition. The seeds were dried to constant wt at 110°. Fresh wt/dry wt ratios were found and the amount of carnitine per g fr wt and per g dry wt calculated.

Incubation medium. The incubation medium contained, in a final vol of 0·5 ml; Pi buffer, pH 6·8, 100 mM; EDTA, Na salt, 1 mM; acetyl-CoA-(1-^{14}C), 25 nCi, 3·86 nmol; acetyl-CoA, 20 μM; 0·1 ml cotyledon extract or standard carnitine soln. The reaction was started by adding 0·1 ml carnitine-*O*-acetyltransferase soln. Incubations were carried out for 30 min at 28°. Controls containing no extract or carnitine were included in every series of assays.

Determination of acetyl-carnitine. After incubation, 0·4 ml of incubation mixture plus 0·1 ml H_2O was pipetted onto columns (3·5 × 0·8 cm) of Dowex 2-X8 (200–400 mesh, Cl^- form). The columns were then washed very slowly with 7 × 0·5 ml fractions of H_2O. All eluate fractions were bulked, the final vol. being 4 ml. The eluate contained acetyl-(^{14}C)-carnitine formed in the reaction and the column retained unchanged acetyl-(^{14}C)-CoA substrate. 0·5 ml aliquots of the eluate were added to vials containing NE260 liquid scintillator, and counted.

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