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SHORT CUMMUNICATION

THIAMINE METABOLISM IN GERMINATING MAIZE SEEDLINGS

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Abstract—Maize seedlings germinated for 10–12 days in either light or darkness show no net synthesis of thiamine. However, after 10 days 50 per cent of the thiamine is in the form of α-hydroxyethylthiamine, which is not present in ungerminated seeds. After about two weeks the thiamine content of the plants begins to increase and this is maintained throughout the growing period. α-Hydroxyethylthiamine is present at all stages except in mature cobs.

INTRODUCTION

DURING our investigations on the biosynthesis of thiamine in yeast,[1,2] it was decided to examine germinating maize seedlings as possible material for the study of thiamine biosynthesis in higher plants. Two aspects were examined (*a*) the nature of the thiamine derivatives in the plants and (*b*) the synthesis of thiamine during various stages of growth. Part of this work has already been briefly reported.[3]

RESULTS

Identification of α-Hydroxyethylthiamine

The purified, de-salted, thiamine-containing extracts of germinated maize seedlings prepared as described in the Experimental section were taken to dryness on a rotary evaporator and the residues extracted with absolute ethanol acidified to pH 4·0 with HCl. The extracts were chromatographed on thin layers of Kieselgel G in a solvent system containing pyridine–isobutanol–water (4:1:1, v/v). The developed chromatograms were sprayed with $K_3Fe(CN)_6$ solution,[4] dried and viewed under the u.v. lamp. Thiamine and α-hydroxyethylthiamine were identified by their positions on the chromatogram compared with authentic samples of these two materials. It was found that both components were present in 3-day germinated seedlings, 10-day seedlings, all green parts and in young cobs. α-Hydroxyethylthiamine was, however, absent from ungerminated seeds and mature cobs.

Quantitative determinations on 10-day seedlings indicated that 50 per cent (50, 48, 51 per cent in three experiments) of the total thiamine was in the form of α-hydroxyethylthiamine.

Thiamine Synthesis in Germinating Seedlings

Total thiamine levels were measured every 24 hr during a germination period of 10 days in the light. Batches of five seedlings were examined and the mean values for five experiments are recorded in Fig. 1, from which it can be seen that little, if any, net synthesis occurs during

[1] D. B. JOHNSON, D. J. HOWELLS and T. W. GOODWIN, *Biochem. J.* **91**, 8P (1964).
[2] D. B. JOHNSON, D. J. HOWELLS and T. W. GOODWIN, *Biochem. J.*
[3] D. B. JOHNSON and T. W. GOODWIN, *Biochem. J.* **88**, 62P (1963).
[4] A. ROSSI-FANELLI, N. SILIPRANDI and P. FASELLA, *Science* **116**, 711 (1952).

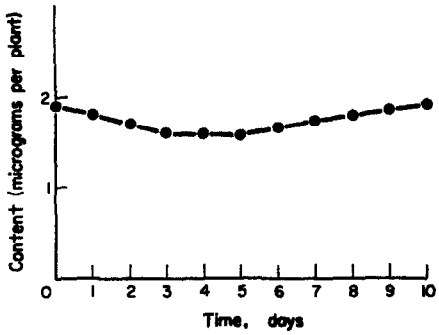

FIG. 1. THIAMINE CONTENT OF MAIZE SEEDLINGS GERMINATED IN THE LIGHT.

germination in the light. Similar experiments with seedlings kept in the dark yielded almost identical results. A further experiment in which seedlings germinated in the dark for 5 days were transferred to the light for a further 5 days indicated no differences between these seedlings and those maintained in continuous darkness (Table 1).

TABLE 1. THIAMINE CONTENT OF MAIZE SEEDLINGS GERMINATED IN DARKNESS FOR 5 DAYS AND THEN KEPT EITHER IN DARKNESS OR LIGHT FOR A FURTHER 5 DAYS

Age of seedlings (days)	Thiamine content (μg/plant)	
	In darkness throughout	Transferred to light after 5 days
6	1·74	1·75
7	1·81	1·80
8	1·92	1·89
9	1·93	1·91
10	2·19	2·21

Experiments were than carried out on growing plants and it was shown that as the plant develops and photosynthesis becomes well established the thiamine content per plant also rapidly increases (Fig. 2).

DISCUSSION

The finding that little if any net synthesis of thiamine occurs during germination of maize seeds is in line with observations made on germinating wheat by Hoffer *et al.*,[5] who found that the thiamine level remained virtually constant over a germination period of 18 days in the dark. In this respect thiamine is different from riboflavine, niacin and pyridoxine, all of which increase during germination. However, the present experiments show that thiamine is not metabolically inert, for after 10 days' germination 50 per cent is in the form of α-hydroxyethylthiamine. As it is thiamine pyrophosphate which is the active co-factor in oxidative decarboxylation, there is little doubt that α-hydroxyethylthiamine pyrosphosphate is the naturally occurring form; this would be dephosphorylated during our extraction procedures.

[5] A. HOFFER, A. W. ALCOCK and W. F. GEDDES, *Cereal Chem.* 23, 76 (1946).

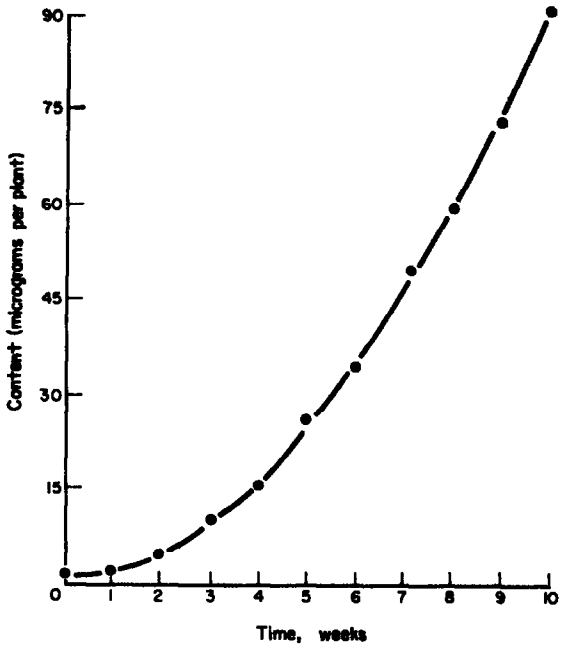

FIG. 2. THIAMINE CONTENT OF MAIZE PLANTS GROWN IN THE GREENHOUSE.

The demonstration of α-hydroxyethylthiamine in germinating seedlings also indicates that thiamine is actively concerned in the metabolic processes associated with germination.

It is only after about two weeks' germination when photosynthesis is well established that thiamine synthesis is well established (Fig. 2). From these investigations it is clear that germinating seedlings are not suitable material with which to study thiamine biosynthesis.

EXPERIMENTAL

Material

Maize (South African Horse Tooth hybrid No. 159c/4221) supplied by Messrs. Gunsons (Seeds) Ltd., London, was used throughout the investigation. Seeds were soaked in water for 24 hr and germinated in moist vermiculite at 28° ∓ 1° in a growth room, either in the light or in light-tight cupboards. Mature plants were grown in earth in 24-in. pots in a greenhouse.

Extraction of Thiamine

Ungerminated seeds. Seeds were cut into a suitable size and milled in a laboratory mill (Glen Creston, Stanmore). An accurately weighed sample was introduced into the tube of a laboratory homogenizer (MSE) and homogenized in 0·1 N HCl (35 ml). The homogenate was transferred to a 100 ml conical flask and the tube and blades washed with 0·1 N HCl, and the washings added to the homogenate. The volume was made accurately to 75 ml and the flask heated on a boiling water bath for 30 min. The extract was cooled and filtered. The residue was washed with dilute HCl (pH 4·5) and the filtrate and washing transferred to a 150 ml conical flask. They were then ready for further treatment.

Germinated seedlings and cobs (maize heads). The procedure for ungerminated seeds was followed except that the milling step was omitted.

Mature plants. Large plants were finely divided with scissors and transferred to a large conical flask (2–3 l.) containing 1·5–2·0 l. of 0·1 N HCl. The material was then homogenized with an Ultra Turrax homogenizer run for 30-sec spells. When homogenization was complete the extraction was continued as just described.

Preparation of thiamine extracts for quantitative determinations. The method was a slight modification of the procedure recommended by the Association of Vitamin Chemists.[6] The thiamine was determined fluirimetrically[6] in an Eel fluirimeter.

Preparation of Extracts for Chromatography

The extract was desalted by the phenol method.[7,8] The desalted extract was taken to dryness in a rotary evaporator; the residue was dissolved in ethanolic HCl (pH 4·0) and made up to a known volume for fluorometric assay; the remaining solution was then taken to small volume for chromatographic analysis.

Chromatography. Thiamine and α-hydroxyethylthiamine were separated on paper chromatograms in three solvent systems[8] (i) *n*-butanol: ethylene glycol: 0·1 N HCl (4:1:1, v/v); (ii) *n*-butanol: acetic acid: H_2O (4:1:5 v/v; upper phase); pyridine: water (4:1, v/v). The spots were visualized in u.v. light (Hanovia Lamp, Model 16, with Chance OX1 filter) as dark absorbing spots; increased sensitivity was obtained by first spraying the chromatogram with $K_3Fe(CN)_6$ solution[3] which converted thiamine and α-hydroxyethylthiamine into their corresponding thiochromes which exhibit bright blue fluorescence in u.v. light. The separation on all these systems takes about 24 hr. A thin layer system was therefore developed which cut the development time to 2 hr. The most effective separation was obtained with Kieselguhr (Merck) as adsorbent and pyridine: isobutanol: water [4:1:1, w/v] as developer. Typical R_f values are thiamine 0·03; α-hydroxyethylthiamine 0·22; thiazole hydrochloride 0·30; pyridine sulphonate 0·70; and thiochrome 0·98.

Quantitative Determination of Thiamine and α-Hydroxyethylthiamine

Aliquots of the prepared extract were chromatographed on the thin-layer system just described. The developed chromatograms were sprayed with oxidizing solution ($K_3Fe(CN)_6$), dried in a current of hot air and examined under u.v. light. The areas of thiamine and α-hydroxyethylthiamine were marked out, and then scraped onto a filter paper in a small funnel and eluted with isobutanol (7 ml). Equal areas of adsorbent from the corresponding areas in a blank region of the chromatogram were treated in an identical manner. With the eluate from the blank area of the plate as blank reference solution and an oxidized solution of thiamine as a standard reference solution, the amounts of thiamine and α-hydroxyethylthiamine in the eluted spots were determined fluorimetrically.[6]

α-Hydroxyethylthiamine. This was synthesized by the method of Krampitz and his colleagues.[9]

Acknowledgement—We are grateful to Shell Research Ltd. for financial support of this investigation.

[6] Association of Vitamin Chemists. *Methods of Vitamin Assay*, New York, Interscience (1947).
[7] J. M. IACONO and B. C. JOHNSON, *J. Am. Chem. Soc.* 79, 6321 (1957).
[8] G. L. CARLSON and G. M. BROWN, *J. Biol. Chem.* 236, 2099 (1961).
[9] L. KRAMPITZ, G. GREULL, C. S. MILLER, J. B. BICKING, H. R. SKEGGS and J. M. SPRAGUE, *J. Am. Chem. Soc.* 80, 5983 (1958).