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The Effect of Zinc Deficiency on the Aldolase Activity in the Leaves of Oats and Clover

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It is now well established that zinc is an essential nutrient element for plants. When the supply of zinc falls short of the physiological requirements of green plants, the retarded growth, the chlorosis, and the morphological changes in the organized structures which supervene, all suggest serious metabolic disturbances. Several biochemical lesions have been described. An abnormal protein metabolism (Bean, 1942), an interruption of tryptophan synthesis and thus of the production of β -indole-3-acetic acid (Tsui, 1948), an accumulation of inorganic phosphorus and an impairment of carbohydrate metabolism (Reed, 1946), have all been observed in zinc-deficient green plants.

In fungi, the retardation of growth in zinc-deficient media has been claimed to be due to a failure of carbohydrate metabolism, in particular, to a break of one or more links in the chain of anaerobic glycolysis (Foster & Denison, 1950). Marked changes in the enzymic constitution of zinc-deficient *Neurospora crassa* have been described by Nason, Kaplan & Colowick (1951).

The accumulation of reducing sugars and the relative shortage of sucrose and starch, which have been observed in the leaves of zinc-deficient tomato plants, imply an impairment of a fundamentally important step in carbohydrate metabolism which might possibly be attributed to a partial failure of aldolase activity (Reed, 1946). There seems little reason to assume, however, that zinc is concerned directly with the aldolase activity in the tissues of green plants, since the activity, *in vitro*, of aldolases from different sources is not invariably influenced by reagents which form un-ionized complexes with heavy metals. As there is no experimental evidence to support the suggestion (Reed, 1946) that the disordered carbohydrate

metabolism in zinc-deficient plants may be attributable to a partial failure of aldolase, the aldolase activity has been estimated in the leaves of normal and of zinc-deficient oats (*Avena sativa* var. Algerian) and of subterranean clover (*Trifolium subterraneum* var. Bacchus Marsh); and the estimations have been extended to include a series of determinations of the aldolase activity in the tissues of oat plants grown in copper-deficient media.*

EXPERIMENTAL

The water cultures. The main salts employed for the nutrient medium were freed from Zn and Cu by the exhaustive extraction of aqueous solutions at pH 7.0 with dithizone in CCl_4 and were subsequently recrystallized. The trace salts were purified by recrystallization.

The procedure adopted for growing the plants was briefly as follows: six plants were suspended through holes in the heavily paraffin-waxed wooden lids that covered each of the series of 3 l. Pyrex beakers containing the aerated culture solutions. Light was excluded from the solution by covering the outsides of the beakers with opaque paper. The solutions were aerated for 1 hr. each day with a stream of air, freed from dust, and saturated, by passing through a glass tower packed with glass Raschig rings immersed in glass-distilled water, and conveyed through plastic tubing. The water lost from the medium by transpiration and evaporation was replaced with glass-distilled water.

Each beaker held 3 l. of a culture solution containing: KNO_3 , 1.0 g.; KH_2PO_4 , 0.5 g.; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25 g.; ferric citrate, 0.04 g.; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 4 mg.; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.25 mg.; H_3BO_3 , 0.57 mg. (all/l.) Zinc sulphate and copper sulphate supplements were added to make the following final concentrations: for the zinc-deficient plants, 100 μg . Cu/l.; for the copper-deficient plants, 200 μg . Zn/l.; for the controls, 100 μg . Cu/l., 200 μg .

* A brief note of the findings has been published (Quinlan-Watson, 1951).

Zn/l. The reaction of the media was approximately pH 5.0. To ensure that iron deficiency would not become a serious limiting factor, further additions of ferric citrate (5 ml. 0.8% ferric citrate/beaker) were made from time to time. The culture solutions were replaced after 50 days.

The oat and subterranean clover seeds from selected graded stocks were soaked overnight in glass-distilled water and then germinated on waxed cotton gauze suspended over glass-distilled water in a saturated atmosphere. The clover plants were transplanted to the water-cultures 10 days, and the oats 14 days, after germination. Plants in the Cu, Zn-supplemented medium grew normally. Deficiency lesions became apparent in the leaves of plants grown in the un-supplemented media within 50–60 days after transplanting. The oat plants were harvested 93–119 days, and the clover plants 141–146 days, after transplanting.

Aldolase estimation. Portions of green leaf showing no visible lesions, see below, were ground with a glass pestle and mortar to which were added 40 ml. of glass-distilled water per g. of green leaf. The suspension was strained through fine gossamer.

The aldolase activity of this suspension was estimated by the method of Sibley & Lehninger (1949), which depends upon the measurement of the colour density ($2 - \log T_{\lambda 540}$) of the 2:4-dinitrophenylhydrazine complex with the trioses formed by enzymic decomposition of hexosediphosphate, further modification of the triosephosphates being prevented by the addition of hydrazine. A portion of the suspension (0.1 ml.) was incubated at pH 8.6 (trihydroxy-methylaminomethane buffer) with the substrate at 38°. After 30 min. the activity was suppressed by addition of trichloroacetic acid and the solutions, now each of total vol. 4.5 ml., were freed from turbidity by centrifuging. Trioses were estimated in 1 ml. portions of the clear supernatants.

The dry matter in the leaf suspension was ascertained by evaporating a 5 ml. portion and drying at 105°.

As the 2:4-dinitrophenylhydrazine complexes of the two trioses, glyceraldehyde and dihydroxyacetone, which are the products of aldolase activity, differ slightly in colour intensity, Sibley & Lehninger (1949) standardized the mixed colour against the alkali-labile phosphate of the resulting phosphotrioses. In the present study no attempt was made to standardize the spectrophotometric evaluation of the trioses, as the primary aim was to estimate the relative aldolase activities of normal and zinc-deficient plant tissues. However, in order to compare the activities (I) found in normal tissues with values reported in the literature, the unit of activity Q/m , employed here, was computed in the following manner.

The alkali-labile phosphorus, P_{Alk} , formed, is directly proportional to $2 - \log T_{\lambda 540}$ of the 2:4-dinitrophenylhydrazine complex (Sibley & Lehninger, 1949). Thus $P_{Alk} = m(2 - \log T_{\lambda 540})$ where P_{Alk} is expressed in $\mu\text{g./ml.}$ supernatant, and m is a constant.

As 1 μmole of hexosediphosphate, HDP, is transformed by aldolase to 2 μmoles of triosephosphates, i.e. to 62 $\mu\text{g.}$ of P_{Alk} , $\frac{m(2 - \log T_{\lambda 540})}{62}$ is a measure of the number of μmoles of HDP (per ml. supernatant) transformed in the reaction.

To convert to 'Q' notation the final spectrophotometric reading of the trioses formed after 30 min. incubation of the leaf suspension with HDP under the defined conditions, the

relative enzyme activities per hour per mg. of dry tissue are expressed by

$$Q_{\text{HDP}} = \frac{4.5 \times 2 \times m(2 - \log T_{\lambda 540})}{62 \times D} \frac{22.4}{\mu\text{l. HDP/hr./mg.}},$$

the factors 4.5 being the vol. of trichloroacetic acid supernatant and D = mg. dry matter in the 0.1 ml. tissue suspension.

As 'm', the constant, was not determined, activity of the preparations reported here are expressed as

$$\frac{Q}{m} = \frac{3.25(2 - \log T_{\lambda 540})}{D}.$$

This provides a measure of the relative activities; the value of m , however, is probably between 6 and 7 when calculated from data supplied by Sibley & Lehninger (1949).

RESULTS

The macroscopic lesions and symptoms exhibited by the deficient plants

Obvious lesions characteristic of the deficiencies became apparent within 50–60 days of transplanting to the zinc-free and copper-free culture solutions.

Avena sativa. The copper-deficient oat plants displayed the white tips, the marginal necrosis, the dark bluish green colour of the leaves, and the marked tendency to tiller that have been observed and described by others (Rademacher, 1936; Riceman, Donald & Evans, 1940; Piper, 1942). Under the conditions imposed vegetative growth was relatively good, but the plants grown in the copper-deficient medium failed markedly to reach the height of their copper-supplemented controls, and they developed, in comparison, about twice the number of tillers.

The zinc-deficient oat plants were seriously dwarfed. Severe marginal necrosis of the leaves became evident, and yellowish-white spots surrounded by bronze-green, purple or brown areas were prevalent lesions. In some leaves these degenerative changes were so extensive that only the area immediately adjacent to the central vein remained green. The younger leaves, on emerging, were softer, paler green, and smaller than those of the zinc-supplemented controls. The material in which the aldolase activity was estimated was selected from the green parts of leaves in which necrosis of the distal portions, and a pale green colour, were the only lesions. Throughout the period of observation there was always a proportion of such leaves on each plant.

Trifolium subterraneum. The runner-internodes of the zinc-deficient clover plants failed to elongate and, because of this, and the fact that many more lateral runners developed, the plants had a closely bunched habit. The younger leaves were small, misshapen and, because of the failure of runner internodes to develop and of the excessive development of lateral runners, densely crowded. It is of interest, here, to recall that the failure of zinc-deficient tomato plants to elongate normally was attributed by Skoog (1940) to lack of auxin production, and that Tsui (1948), when investigating this phenomenon, showed that under these conditions the production of tryptophan, the probable precursor of the auxin, was materially reduced, and he concluded that zinc is involved in tryptophan production. Recently Nason (1950) and Nason *et al.* (1951) have demonstrated that the activity of the enzyme which catalyses the condensation of indole and

serine to form tryptophan is greatly decreased in the mycelium of *Neurospora crassa* grown in zinc-deficient media, and that although mycelial growth was stimulated by the addition of a complete mixture of amino-acids to the medium, the activity of this enzyme was not enhanced.

In addition to the striking alteration of the habit exhibited by the zinc-deficient subterranean clover plants, necrotic patches appeared on the older leaves and, in some cases, extended throughout the whole leaf.

Aldolase activity

Only leaves or portions of leaves in which there were no obvious lesions were assayed for aldolase activity. The aldolase activities and some estimations of the zinc and copper contents of the leaves from the zinc- and the copper-deficient plants and of their supplemented controls are reported in Tables 1 and 2. From these figures it is obvious that zinc-deficiency, but not copper-deficiency, led to a considerable decrease of the aldolase activity in the leaves of both oat and subterranean clover plants.

Table 1. *Aldolase activity and Zn + Cu contents of oats (Avena sativa var. Algerian) cultured in Zn- or Cu-deficient media*

Description		μg./g. dry wt. of leaf		Aldolase of leaf Q/m
		Cu	Zn	
Control	1	6.2	39.2	6.6
	5	4.4	28.8	8.1
	5	5.55	32.8	8.4
	9	5.35	23.0	5.4
	9	5.8	24.2	8.3
	Av.	5.46	29.6	7.4
Cu-deficient	4	1.4	39.2	7.3
	6	1.15	33.6	7.1
	6	1.15	30.0	7.0
	3	2.4	10.8	6.2
	3	2.1	10.2	6.0
	Av.	1.64	24.8	6.7
Zn-deficient	7	12.8	8.6	0.95
	8	9.5	8.2	1.3
	10*	3.1*	10.4*	4.6*
	Av.	—	9.1	2.3

* Both copper- and zinc-deficient.

Table 2. *Aldolase activity in leaf of subterranean clover (Trifolium subterraneum var. Bacchus Marsh)*

Aldolase activity [Q/m] of leaves	
Control	Zn-deficient
2.4	0.42
2.2	0.28
1.9	0.32
2.2	0.34

DISCUSSION

In the absence of an alternative channel of carbohydrate metabolism distinct from the customary

course via the hexose- and triose-phosphates, any considerable decrease of aldolase activity might be expected to limit materially the rate at which the reversible transfer of phosphoglyceraldehyde to fructose-1:6-diphosphate may be accomplished, and so to impair seriously not only the production of hexoses during photosynthesis but also the exergonic glycolytic reactions. Thus it is not improbable that the reduction of the rate of carbohydrate metabolism by a partial failure of one of the intermediary enzymes is a biochemical lesion of fundamental importance in zinc-deficient green plants, for, in both species of plants studied, zinc deficiency led to a considerable decrease of the aldolase activity in the green leaves.

Three among a number of possible explanations of this phenomenon appear most plausible: zinc may be a constituent of the apo-enzyme, it may be directly or indirectly concerned with the synthesis of proteins or more specifically with the synthesis of aldolase itself, or it may function in association with, or as, a co-enzyme.

There is no direct evidence to support the first of these possibilities. The aldolase activity of yeast and of *Clostridium perfringens* has been reported to be inactivated by potassium cyanide, etc. Zinc, however, has no specific capacity to reverse these inhibitory effects; Cu^+ , Fe^{2+} , Co^{2+} share with Zn^{2+} the capacity to reverse the inhibition of yeast aldolase by potassium cyanide, cysteine, pyrophosphate, and 2:2'-dipyridyl (Warburg & Christian, 1943), and similar inhibition of the aldolase of *Cl. perfringens* is reversed, apparently, only by Fe^{2+} and Co^{2+} (Bard & Gunsalus, 1950). Moreover, the aldolase of *Escherichia coli* has been reported to require Mn^{2+} specifically for full activity (Knox, Stumpf, Green & Auerbach, 1948). Little is known as yet of the behaviour, *in vitro*, of the aldolases from the higher plants under these conditions. Aldolase activity is clearly widely distributed in green leaves (Tewfik & Stumpf, 1949), but partially purified aldolase from pea seeds (Stumpf, 1948) like the aldolases from rabbit muscle (Herbert, Gordon, Subrahmanyam & Green, 1940; Taylor, Green & Cori, 1948) or from rat muscle (Warburg & Christian, 1943) is apparently not activated by heavy metals.

The second possibility is contentious. It is not clear whether the retarded rate of synthesis of both protein and starch in incipiently zinc-deficient tomato plants, described by Bean (1942), is the result of a primary or of a secondary effect of the deficiency; and in view of the widely divergent effects of zinc deficiency on individual enzymes in *Neurospora crassa*—in the zinc-deficient mycelium of this organism the activities of the alcohol dehydrogenase and of a tryptophan-synthesizing enzyme are markedly decreased, the activity of aldolase and other enzymes are unchanged, and the

activity of diphosphopyridinenucleotidase is greatly increased (Nason *et al.* 1951)—there is no reason to attribute invariably the disorders of metabolism which occur in zinc deficiency to a primary impairment of protein synthesis. Copper deficiency has been claimed to have marked effects on the protein metabolism of green plants (Gilbert, Sell & Drosdoff, 1946; Wood & Womersley, 1946; Lucas, 1948; Gilbert, 1951), and so the observation that the aldolase activity in the leaves of copper-deficient oats remains unimpaired, suggests that the lowered aldolase activity in the leaves of zinc-deficient oats is not due primarily to a decreased protein synthesis; although zinc might be specifically involved in the synthesis of the enzyme itself.

The third possibility that the aldolases of green leaves are activated by Zn^{2+} or by zinc-containing co-enzymes is being examined.

SUMMARY

Aldolase activity was found to be materially decreased in tissue suspensions prepared from the leaves of zinc-deficient plants of *Trifolium subterraneum* and of *Avena sativa*, but was unchanged by copper deficiency.

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The Deoxyribonucleic Acid Content of the Rat Cell Nucleus and its Use in Expressing the Results of Tissue Analysis, with Particular Reference to the Composition of Liver Tissue

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In 1948, Boivin, Vendrely & Vendrely published figures for the deoxyribonucleic acid (DNA) content of the cell nucleus of vertebrates, based on the chemical analysis of a known number of isolated

nuclei, and claimed that the amount of DNA per nucleus was constant for the somatic cells of different tissues and was approximately double that found in the haploid sperm cells. On the other hand, a considerable variation occurred between species, birds and fish having, in general, a lower DNA content per nucleus than mammals (Vendrely & Vendrely, 1948, 1949). The general trend of these

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