mines have been compared for their ability to be oxidized by the extracted enzyme:

$NH_2{\cdot}CH_2{-}CH_2{-}CH_2{-}CH_2{-}CH_2{\cdot}NH_2$	cadaverine
$NH_2{\cdot}CH_2{-}CH_2{-}SS{-}CH_2{-}CH_2{\cdot}NH_2$	cystamine
$NH_2{\cdot}CH_2{-}CH_2{-}S{-}CH_2{-}CH_2{\cdot}NH_2$	lanthionamine
$NH_2{\cdot}CH_2{-}CH_2{-}SO{\cdot}SO{-}CH_2{-}CH_2{\cdot}NH_2$	cystamine disulfoxide
$NH_2{\cdot}CH_2{-}CH_2{-}SO_2{-}CH_2{-}CH_2{\cdot}NH_2$	lanthionamine sulfone

The reaction was followed at pH 5·6 in order to avoid dismutation of cystamine disulfoxide.

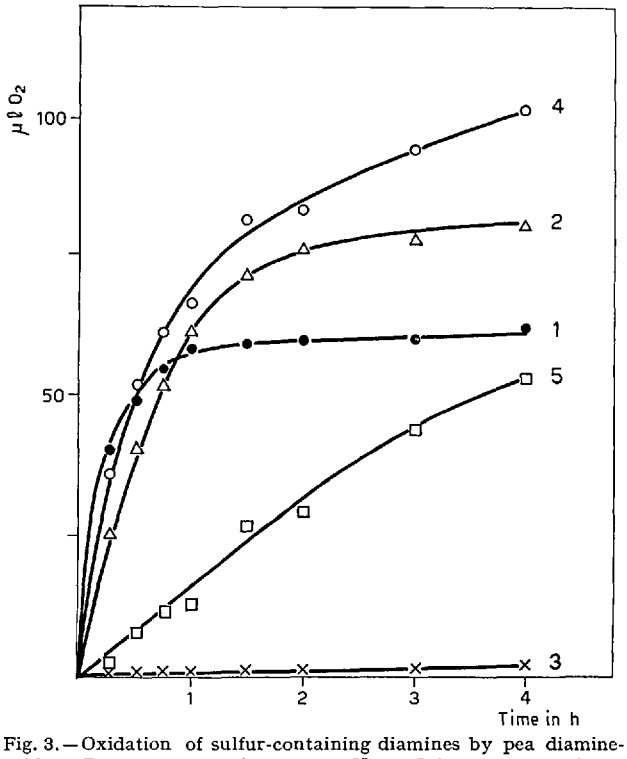

Fig. 3. – Oxidation of sulfur-containing diamines by pea diamine-oxidase. Enzyme preparation 20 mg. pH 5·6. Substrates 5 μM. Temperature 25° C. Other conditions as in Figure 1. *1* Cadaverine; *2* cystamine; *3* cystamine disulfoxide; *4* lanthionamine; *5* lanthionamine sulfone.

As reported in Figure 3, lanthionamine represents the best substrate among the sulfur-diamines. The presence of oxidized sulfur in the molecule results in a depression of activity, in the case of lanthionamine sulfone, and in a complete abolition of activity, in the case of cystamine disulfoxide.

Experiments are continued along this line in order to detect the final products and to relate these findings with the metabolism of cystamine *in vivo*.

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Institute of Biological Chemistry, University of Rome, June 28, 1956.

Riassunto

Le diamine solforate cistamina e lantionamina sono ossidate con velocità comparabile a quella della cadaverina dalla diamino-ossidasi. Il solfone della lantionamina è ossidato più lentamente ed il disolfossido della cistamina non è ossidato affatto. Benchè dall'ossidazione della cistamina si produca una mole di ammoniaca per mole di substrato, il consumo di ossigeno è due volte più elevato del teorico, indicante una ulteriore ossidazione del prodotto metabolico della reazione. Tuttavia taurina, ipotaurina e solfati, non sono determinabili nel deproteinizzato finale.

The Influence of Pyridoxine on the Free Amino Acids of Decotylized Pea Seedlings

In other (unpublished) investigations, it was established that cotyledon-less pea seedlings (if the excision of cotyledons carried out at the 3rd day of germination) are deficient in free glutamic acid, at least for a few days.

According to Burkholder and McVeigh[1] and Cheldelin and Lane[2], an accumulation of vitamin B_6, pyridoxine, is observable in the germinating seeds. The pyridoxine and its several derivatives function as cotransaminases, and consequently play an important role in the intensive transamination of the seedlings[3]. Wilson *et al.*[4] have prepared from 6–10 day old seedlings an enzyme fraction, which, in the presence of pyridoxamine or pyridoxal-5-phosphate, transfers the amino groups to α-ketoglutaric acid.

Fries[5] showed that cotyledon-less pea seedlings require in embryo culture a significantly high amount of pyridoxine.

I have confirmed experimentally in the following manner. Seedlings of "Folger" pea were deprived of their cotyledons after 3 days of germination (at room temperature) and exposed on light in Knop solution. 3 days after, one group of the plants was infiltrated in vacuum (two times during 24 h) with 0·1% pyridoxine dissolved in water. The other group (control) was given only distilled water (also by vacuuminfiltration) at the same time. 24 h later I analyzed the seedlings of the two variants with respect to their free amino acids, using the method of Awapara[6]. The solvent of the (25 cm diameter) paper chromatograms was butylic alcohol–water–acetic acid (4:1:5). The fresh weight of the extracted plant material was of 0·5 g.*

The results of the investigation showed that while the water-infiltrated plants were deficient of free glutamic acid, in the plants provided with pyridoxine glutamic acid appeared also among the other free amino acids (arginine, serine, tyrosine, leucine),

It is probable that in cotyledon-less pea seedlings there is no sufficient amount of pyridoxine.

L. Martos

Chair of plant breeding, High School of Horticulture, Budapest, Hungary, June 18, 1956.

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* The reproducibility of these results was good and free of controversy. The investigation was carried out three times, each of them contained 8 repetitions uniformly showing the results presented above.

Zusammenfassung

Es wird festgestellt, dass in jungen Erbsenkeimlingen, die ihrer Kotyledonen beraubt wurden, einige freie Aminosäuren fehlen. Wenn solche Keimlinge mit Pyridoxinlösung infiltriert werden, ist jedoch unter ihren freien Aminosäuren auch die sonst fehlende Glutaminsäure nachweisbar.

Utilization of iron from Ethylenediamine Tetraacetic Acid-Iron Chelate Complex in Culture Medium by Fusarium

The need for this investigation arose while the writers were engaged with the problem of the role of micronutrients in fungi, especially in the reduction of nitrates and sulphates by *Fusarium lycopersici* Sacc. (strain ETH 5414, kindly supplied to us by Dr. H. KERN of the Swiss Federal Institute of Technology, Zurich). When iron as $FeCl_3 \cdot 6\ H_2O$ was added to the purified Richard's medium, it invariably led to the precipitation of the insoluble ferric phosphate even at pH 4·0. The only possibility that suggested itself was the use of some chelating agent in the artificial culture medium to check the formation of the precipitate. Some workers on microbial nutrition[1] have used hydroxycarboxylic acids as the chelating agents, but this procedure has been criticized[2] on the ground that such acids are regular participants in the fungal metabolism. SCHATZ and HUTNER[2] have recommended the use of ethylenediamine tetraacetic acid (EDTA) in this connection. EDTA forms soluble chelate complexes with many metallic ions, e.g., Fe^{++}, Ca^{++} and Mg^{++} etc.[3]. While EDTA has found excellent usage as a chelating agent for nutritional work with higher plants, algae[4] etc., and seems to be biologically inert for them, no irrevocable proof of its inertness is available as regards the fungi. REISCHER[5] has used EDTA as a chelating agent in his work on the inorganic nutrition of Saprolegniaceae without any adverse effects. FRIES[6], however, finds EDTA toxic for some species of *Coprinus* in artificial culture, specially in pH ranges below 7. Before using the chelating agent, therefore, its effect on the growth of *Fusarium* had to be ascertained.

Fusarium lycopersici was grown in Richard's medium in 2 series of Erlenmeyer flasks of 150 ml capacity. Each flask contained a total of 20 ml of the nutrient solution. In one series of flasks 5 mg of iron per l as iron-EDTA complex was added (for preparation see ARNON *et al.*[4]) and in the other series an equivalent amount of iron as $Fe_2(SO_4)_3 \cdot 7\ H_2O$ was included. Apart from iron, all the flasks received an optimal quantity of a micronutrient solution[7]. Glass (Pyrex) redistilled water was used in making solutions and the latter were adjusted to a pH of 4·0 before autoclaving. The flasks received a uniform amount of conidial suspension raised in rice cultures[8]. They were incubated in the dark at 25°C. After every second day of growth, replicate flasks from each series were taken, acidified with redistilled HCl to a pH of 2·0 (to dissolve any precipitated iron phosphate) and filtered through a weighed Whatman filter no. 40. The mycelium was washed with 50 ml of acifidied redistilled water and the filter paper along with the mycelium was dried overnight at 60°C and finally at about 105°C for a further period of 3 h. These were then weighed in a balance. Total iron in the mycelium was determined by using the α-α′-dipyridyl reagent[9]. A weighed quantity of the mycelium was ashed in a crucible using a drop of iron-free HNO_3 towards the end. The residue was extracted with $N \cdot HCl$ solution and iron determined in this using a 10% solution of sodium sulphite to reduce the Fe^{+++} to Fe^{++} form. The pink colour was read off in a Spekker photoelectric colorimeter with the help of a green filter (maximal absorption near 520 μm) and the amount of iron estimated with the help of a standard curve.

Days	Total dry weight/ml medium		μg Fe/mg mycelium dry weight	
	with EDTA	without EDTA	with EDTA	without EDTA
	mg	mg		
3	3·5	3·3	0·046	0·036
5	5·0	3·5	0·027	0·030
7	7·0	7·0	0·030	0·034
9	8·0	7·3	0·042	0·039
11	9·1	9·0	–	–

The Table above summarizes the results of our investigation. It will be noticed that cultures with EDTA show a consistently better growth than those without the chelating agent. The total amount of iron in the mycelium, however, is nearly the same in both the series and within the range of experimental error. It is clear that EDTA in the amounts used here is not at all toxic to the fungus, and in fact it supports slightly better growth of the organism, perhaps because EDTA does not allow the iron ions to precipitate as the insoluble iron phosphate and thus maintains a constant supply of the micronutrients in the medium in a soluble form.

When *Fusarium lycopersici* is growing on nitrate as the sole source of nitrogen in the medium, the pH of the culture solution rises as the nitrate ions are assimilated[10]. When maximum growth has taken place on the Richard's medium, the pH reaches about 7·0. EDTA is undissociated or partly dissociated at pH values below 7 and the cause of its toxicity has been correlated[6] to the undissociated EDTA in the medium used for culturing certain *Coprinus* species. In the organism which we have used for experimentation, however, such toxicity is not noted at pH values between 4·0 and 7·0. It is quite possible that different fungi behave differently with regard to the presence of EDTA. This is being further investigated in our laboratories.

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