heterocystous condition, which is obtained by growing these in complete absence of combined nitrogen, is due to the excessive leaching of the amino-acids/peptides to the external medium as reported by others[6,10], or due to the limited synthesis of amino-acid in presence of elementary nitrogen which is enough to support only growth and protein synthesis, or to both interplaying simultaneously, is not known.

That heterocystous stages are accompanied by changes in amino-acid composition has been confirmed. But in different forms the changes are different and in some cases opposite to the other species. It is, therefore, not possible to assign much significance to the qualitative changes which take place in the amino-acid composition with heterocyst formation. This may be probably related to the differences in the intermediary metabolism contributing to nitrogen fixation in different forms.

Zusammenfassung. N_2-fixierende Blaualgen wurden unter Bedingungen gezüchtet, die einerseits zu Heterocysten führten, andererseits jedoch diese Bildung verhinderten. Die Analyse der freien Aminosäuren ergab sowohl qualitative als auch quantitative Unterschiede, ohne dass ein eindeutiger Zusammenhang zwischen der Synthese einzelner Aminosäuren und dem Vorhandensein beziehungsweise der Abwesenheit von Heterocysten beobachtet werden konnte.

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Effect of Methyl Phenyldiazenecarboxylate (Azoester) on the Germination of the Fungus *Trichoderma viride*

Certain stages of cell division apparently depend upon adequate intracellular levels of thiols[1,2]. These thiols are of low molecular weight and probably consist largely of glutathione (GSH). For present purposes, we shall discuss experiments in terms of GSH or thiols of equivalent reactivity.

A new set of reagents has been introduced recently for the intracellular oxidation of GSH to GSSG[3-5]. One of these reagents, methyl phenyldiazenecarboxylate (azoester), reacts with GSH according to the stoichiometry shown in eq. (1).

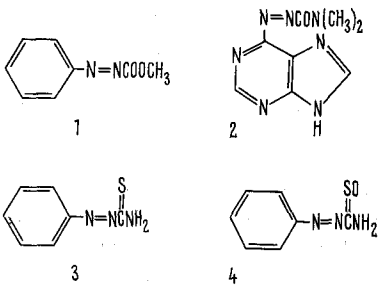
$$2GSH + C_6H_5N{=}NCOOCH_3 \rightarrow GSSG + C_6H_5NHNHCOOCH_3 \quad (1)$$

The complete chemistry of azoester is discussed elsewhere[5]; we must here only point out that a side reaction, hydrolysis, leads to the formation of free radicals which cause intracellular damage. The damage, however, ensues only if the intracellular GSH has been diminished to a very low level.

We have utilized azoester to investigate whether or not GSH was more essential at some stages of germination of the fungus *Trichoderma viride* than at others. RICHMOND and SOMERS[6] have shown that soluble thiols (presumably GSH) increase more rapidly than dry weight or extent of germination in the fungus *Neurospora crassa*.

Spores of *T. viride* were added to minimal media[6,7] to start the germination process. At various times, a $3 \times 10^{-3} M$ solution of azoester was added to the spore suspension in sufficient quantity to make a final azoester concentration of $1 \times 10^{-3} M$. The pH of the medium was between 5 and 6, and the half-life of the azoester was about 30 min under these conditions. Thus, within 1–2 h, the azoester concentration had been reduced to a point where control experiments indicated that it had no effect of any kind. The extent of germination was evaluated by counting under a low power microscope: any spore with an emerging filament was counted as germinated. The results are shown in the Table. Controls are carried out by mixing water with spore suspension and recording the extent of germination at 22 h after initiation. At least 95% of the control spores germinated.

The results indicate that successful germination is prevented or severely delayed by intracellular oxidation of GSH. Further experiments with a thiol-oxidizing agent which does not generate free radicals, 6-purinyldiazenecarboxylic acid N, N-dimethylamid, demonstrated that the destructive action of the free radicals was the



Effect of azoester on germination of fungus *Trichoderma viride*

Time of azoester treatment[a] (h after start)	Extent of germination at + 22 h[b] (% of control)
0	100
4	50
8	3
14	1–2
18	33

[a] Azoester has a half-life of 20 min in neutral aqueous solution.
[b] Time after exposure of spores to minimal media.

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[7] Minimal medium contains 0.5 g $MgSO_4$, 1.0 g KH_2PO_4, 10 g glucose and 0.5 g DL-alanine in 1000 ml distilled water, pH 5–6.

process which prevented germination, since the 'innocuous' agent only delayed germination slightly when used at the same concentration level as azoester.

We have also found that azoester inhibits growth and sporulation. Inocula of *T. viride* on filter paper were allowed to grow on minimal media for 24 h. The papers were transferred to an azoester solution ($10^{-3}M$), allowed to remain in the solution for 1 h, transferred again to fresh minimal media, exposed briefly to light to stimulate sporulation, and allowed to stand for 24 h in the dark. Growth was markedly inhibited (10–20% of controls) and sporulation was almost completely abolished.

We may thus conclude that glutathione is somehow involved in those processes which result in germination, growth, and sporulation. The use of thiol-oxidizing agents for the investigation of the role of GSH may also aid in elucidating some characteristics of the processes of germination, growth, and sporulation.

It is interesting to compare the structures of the intracellular thiol-oxidizing agents we have discovered (**1** and **2**) with those of the recently reported fungicidal agents **3** and **4**[8].

Résumé. Le traitement des spores de *Trichoderma viride* avec le nouveau oxydant intracellulaire des thiols, le composé azoester, nous a conduit à conclure qu'il y a une exigence absolue pour le glutathion pendant le processus de germination. Avec le même composé, azoester, on a demontré que GSH est nécessaire pour la croissance et la sporulation du champignon.

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[11] This research was supported in part by a U.S.D.A. grant under U.S. Public Law No. 480.

Influence of Dormancy on the Chemical Composition of *Strophocheilus* (Strophocheilidae, Pulmonata, Mollusca)

Physiological and biochemical changes during hibernation and estivation in pulmonates have been investigated by several authors, chiefly with regard to *Helix pomatia*; information on the subject has recently been summarized by Hyman[1]. For representatives of the suborder *Mesurethra*, no information is available. This group is extensively represented in the neo-tropical region by the genus *Strophocheilus*, family *Strophocheilidae*, in relation to which some work has already been done concerning its biology and distribution[2,3]; data on the physiology and chemical composition of different organs and tissues have recently been provided[4,5]. Specimens of *Strophocheilus* are known to go into a state of dormancy, either during hibernation or estivation; such behaviour allowed the authors to carry out the present investigation, with the aim of determining the influence of both hibernation and estivation on the chemical composition of the entire body, without shell, of the snail *Strophocheilus oblongus musculus*.

Material and methods. Specimens of *Strophocheilus* were collected in the neighbourhood of Porto Alegre, Rio Grande do Sul (Brazil) and sent to São Paulo, where they were kept in the terrarium of the Zoology Department, University of São Paulo. 3 groups of 10 animals each were studied: one consisted of active animals, kept at 23–24 °C, the other of hibernating animals at 16–17 °C for 45 days, and finally a third group of estivating animals kept at 31–32 °C for 45 days. During this period, relative humidity ranged from 60–80%. All these animals had been, 10 days previous to the experimental procedure described, subdivided in 2 groups, 1 fed with lettuce and the other with cabbage, these plants being known to contain different amounts of calcium and protein[5]; no differences, however, were detected in the chemical constitution of animals kept under different diets, results being pooled for discussion.

At the appropriate time, the animals were sacrificed and the shells carefully removed to avoid any possibility of contamination, especially in relation to calcium. Entire animals, without the shell, were weighed and then dried in an oven regulated at 100 °C, until constant weight was obtained; these remains were then carefully homogenized in a porcelain mortar. The contents of water, sodium, potassium, calcium, magnesium, phosphorus, sulphur, copper, iron, nitrogen, glycogen and total carbohydrates were determined in active animals, as well as in those under estivation or hibernation. Determinations of glycogen and total carbohydrates were necessarily carried out with fresh material. The analytical methods used have already been described in previous papers[4,6,7]; the results were subjected to conventional statistical treatment.

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