break down of lipids in the liver. Although useful information may be obtained by the analysis of blood lipoproteins and free fatty acids it is considerably more common to determine the levels of cholesterol, phospholipid and total lipid. It is recognized that these three chemical classes of lipids may each be made up of components from many lipid complexes of plasma. Since the blood levels of these lipids and proteins are often of clinical significance and since they are controlled by their levels in liver they are estimated in the livers and serum of test animals. The biosynthesis of triglycerides and phospholipids are dependent on the production of D-1, 2 diglyceride which occurs primarily in the liver and also which requires the participation of CoA. Allicin is ascribed with the property of combining with –SH group, the functional part of CoA which is necessary for the biosynthesis of fatty acids, cholesterol, triglycerides and phospholipids. The lipid lowering effect of allicin may therefore be attributed to its capacity to inactivate –SH group compounds. The cholesterol lowering effect of allicin is more pronounced on the free cholesterol levels than on the total cholesterol levels. The present results are in agreement with the findings of TEMPLE[12] who studied the cholesterol lowering effects of polysulphides resembling those found in garlic oil. The noted decrease in the level of free cholesterol may suggest that its esterification is accelerated and thus the transport and utilization of lipids enhanced. From a quantitative stand point, the serum cholesterol largely if not exclusively arises from the hepatic synthesis. Hence the primary effect of allicin may be on the liver. In a recent paper PRASANNAN[22] has reported that the liver lipid of rats reaches its peak level when they are 5–6 months old. In our study as we used rats of this age group we can state that allicin in some way prevents this fat accumulation in liver. From the results of the present experiment we may presume that the biosynthesis of cholesterol and other lipid components were inhibited in rats fed allicin as this compound can inactivate –SH groups. This may explain the therapeutic values of garlic which is used in the treatment of heart disease and arteriosclerosis. Detailed study on the mechanism of action of allicin is under progress.

*Zusammenfassung.* Experimenteller Nachweis eines hypolipidämischen Effektes des Knoblauch-Inhaltstoffes Allicin nach Langzeit-Fütterungsversuchen mit Ratten, wobei die Allicin-Wirkung sich stärker auf die Leber als auf das Blutserum auswirkt.

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## Cycloheximide: A Specific Inhibitor of Protein Synthesis and Intercellular Ion Transport in Plant Roots

In a recent publication[1], we have provided evidence suggesting that cycloheximide (CHM) specifically inhibits ion transport from an external solution through the root into the root xylem, from where ions are delivered to the shoot. This kind of transport requires movement of ions in the symplasmic continuum which, by way of the plasmodesmata, extends from cell to cell in the root parenchyma[2]. By contrast, ion accumulation in the vacuoles of the root cells, which is largely under the control of membrane transport (plasmalemma, tonoplast), is not impaired by CHM. Since incorporation of $^{14}$C-leucine into protein, but not $^{14}$C-leucine uptake into the root is also strongly inhibited by CHM, we concluded that concurrent protein synthesis is a basic requirement of symplasmic transport. These earlier results are summarized by the following tabulation, in which the values given represent % of the controls obtained in the absence of CHM:

| | CHM 1 | [µg × ml$^{-1}$] 10 |
|---|---|---|
| Ion transport through the root | 60 | 10 |
| Ion accumulation in the root | 98 | 105 |
| $^{14}$C-leucine incorporation into protein | 61 | 38 |
| $^{14}$C-leucine uptake by the root | 100 | 113 |
| Respiratory $O_2$ uptake by the root cells | 104 | 95 |

It has been argued repeatedly, however, that such results have to be considered with extreme care, because antibiotics such as CHM may inhibit protein synthesis rather indirectly and unspecifically, e.g. via impairing energy transferring systems[3–5]. The negative effect of CHM on $O_2$ uptake shown in the above tabulation is not sufficient evidence to rule out an (uncoupling) effect on oxidative phosphorylation. In principle, using inhibitors which may under certain circumstances, but not generally, exert specific effects in systems as complex as the intact plant root, it is not sufficient to rely on the description of inhibitor effects in the literature. A number of control experiments must be performed with the given material and experimental conditions. To support the above conclusion on the specific action of CHM in barley roots, we have compared the effects of CHM on $O_2$ uptake and levels of ATP and ADP in the roots with those of the well-known uncoupler CCCP (carbonylcyanide m-chlorophenyl-hydrazone).

Roots from barley plants grown for 6 days in the dark at 25 °C in Hoagland's culture solution were harvested, rinsed and kept for 2 h in aerated solutions as usually used for the ion uptake and transport experiments (i.e. 5 m*M* KCl + 0.1 m*M* $CaSO_4$; cf. ref. [1]) with CHM added as indicated in the Table. At the end of this period, the tissue was rapidly frozen in liquid $N_2$ and transferred to a

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Respiratory $O_2$ uptake, ATP- and ADP-levels in barley roots with no additions (controls) and with CHM and CCCP respectively added to the medium (5 m*M* KCl+0.1 m*M* $CaSO_4$)

| Medium | Respiratory $O_2$-uptake ($\mu$mole $g_{FW}^{-1}h^{-1}$) | ATP level (nmole $g_{FW}^{-1}$) | ADP level (nmole $g_{FW}^{-1}$) |
|---|---|---|---|
| Control | 10.2 ± 0.3 (28) | 32.2 ± 1.0 (11) | 14.3 ± 1.6 (3) |
| 1 $\mu$g CHM/ml | 11.2 ± 0.5 (6) | 43.1 ± 1.8 (6) | — |
| 10 $\mu$g CHM/ml | 8.3 ± 0.3 (24) | 44.5 ± 1.3 (11) | 22.1 ± 1.7 (3) |
| 2 $\mu$M CCCP | 16.5 ± 0.5 (16) | 3.8 ± 0.2 (9) | 7.0 ± 0.8 (3) |

Errors are standard errors, number of replicates in brackets. FW = fresh weight.

mixture of methanol : chloroform : 7 *M* formic acid (12:5:3, v:v:v) at −25°C. After about 24 h, extraction was performed at 0°C in this solution and with subsequent washings according to BIELESKI[6,7]. ATP and ADP were tested with the luciferase test (firefly lantern extract from Sigma Chem. Comp., St. Louis, Mo., USA) as described by PRADET[8] and as used earlier in our laboratory[9]. $O_2$ uptake was determined polarographically in a Rank-$O_2$-electrode (Rank Brothers, Bottisham, Cambridgeshire, England), where CCCP was added directly to the incubation vessel and $O_2$ uptake measured over a period of 10 min.

The results are compiled in the Table. It is quite evident that the CHM treatments do not lead to reduced ATP levels but may rather somewhat increase the ATP levels in the root tissue. This might be related to reduced ATP consumption by protein synthesis, which is inhibited by CHM (tabulation above), while simultaneously respiration is unimpaired. The small decrease of $O_2$ uptake observed with 10 $\mu$g CHM/ml is not significant (see also tabulation above). Hence, under the conditions of our experiments, CHM has minimal effects on energy transfer (i.e. respiratory $O_2$ uptake and ATP levels) in barley root cells. The last line of the Table provides a test of the immediate response of the root cells to metabolic inhibition. The uncoupler CCCP significantly increases respiratory $O_2$ uptake and drastically reduces the ATP level. ADP levels have been measured only in a few cases. The decrease of ADP levels with CCCP and the increase with 10 $\mu$g CHM/ml is consistent with a considerable decrease of energy charge[10] in CCCP and a small increase in CHM treatments respectively. AMP levels were not determined.

In conclusion it appears possible to use CHM as a specific inhibitor of protein synthesis and associated phenomena such as membrane turnover under experimental conditions where the appropriate control experiments are negative. The new data presented in the Table strongly support our earlier conclusion[1] that some degree of membrane turnover, and therefore of protein synthesis, must be maintained during symplasmic transport of ions in plant tissues. BRINCKMANN[11] has shown recently that membrane potential oscillations of green leaf cells, which are triggered by switching on or off non-cyclic photosynthetic electron flow, are prevented by CHM (10 $\mu$g/ml), although electron flow itself is not impaired (i.e. photosynthetic $O_2$ evolution unaltered). This emphasizes that communication via the cytoplasmic phase is blocked by CHM, since in the case of the membrane potential oscillations such communication would be required between the chloroplasts generating the electrical signal and the electrode (within the cell, presumably in the vacuole) picking it up. Thus CHM seems to have a membrane-active role, which, in view of its ineffectiveness on ion accumulation across plasmalemma and tonoplast in root cells, may be located within the cytoplasm. Cycloheximide may act on membrane turnover of such compartments as the cisternae of the endoplasmic reticulum and, possibly, this is how it inhibits protein synthesis.

*Zusammenfassung.* Cycloheximid wirkt in Gerstewurzeln als spezifischer Hemmstoff der Proteinsynthese und des vermutlich an fortlaufende Proteinsynthese und Membran-Turnover gebundenen Ionentransportes durch die Wurzel in das Xylem. Die respiratorische $O_2$-Aufnahme wird durch Cycloheximid nicht beeinflusst, das ATP-Niveau im Gewebe wird geringfügig erhöht. Unter gleichen Bedingungen steigert der Entkoppler CCCP die $O_2$-Aufnahmerate und senkt das ATP-Niveau drastisch.

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[12] Supported by Deutsche Forschungsgemeinschaft through a research grant to U.L. and a visiting professorship to M.G.P. We are grateful to Miss G. Weber for technical assistance.
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