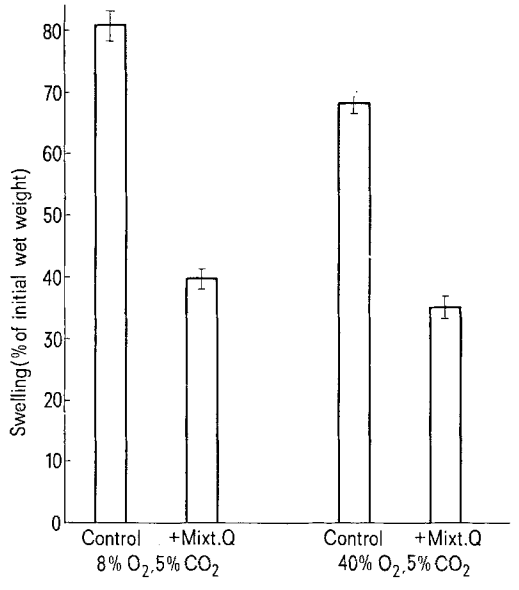
tions. From these data the osmotic changes after addition of mixture Q were calculated, and an equivalent amount of NaCl was subtracted from the K-solution in the experiments of Table II (see also Figure). In consequence, the osmotic pressures were the same in the controls and in the presence of mixture Q and only 43 m*M* NaCl (Figure).



Reduction of the swelling of brain slices by mixture Q. Mean values ± S.E.; $N = 8$ for both series of experiments. Series 1.: Control 106.0 m*M* NaCl, 273.0 ± 0.1 mOsm · l^{-1}; + mixture Q 43.3 m*M* NaCl, 273.0 ± 0.3 mOsm · l^{-1}. Series 2.: Control 106.0 m*M* NaCl, 272.0 ± 0.1 mOsm · l^{-1}; + mixture Q 42.6 m*M* NaCl, 270.6 ± 0.3 mOsm · l^{-1}.

In the experiments of Table II, the glucose consumption, respiration and CO_2 production were stimulated. The degree of stimulation of the O_2 uptake depended on the O_2 supply, whereas stimulations of lactate and of pyruvate formation were almost the same under normal and hypoxic conditions.

As a consequence of the stimulation of the energy-yielding glucose metabolism, the swelling of the brain slices was reduced in both series of experiments to 50% of the control values. It was mainly the intracellular space which decreased, since the inulin spaces remained constant in the presence of mixture Q. Probably the effects contain also a certain component of 'chloride effect' analogous to BOURKE's findings with isethionate[6]; but just the experiments with succinate plus fructose diphosphate and glycerate phosphate are the reason to stress the metabolic aspect of the observed effects.

Zusammenfassung. Es wurde geprüft, ob die Wasseraufnahme von Kaninchen-Hirnschnitten durch Zugabe bestimmter Substanzen zur Inkubationslösung auf nicht-osmotischem Weg verringert werden kann. Bei Einwirkung einer Mischung aus Succinat, CDP-Cholin, Fruktosediphosphat, Phosphoglycerat, Lactose oder Saccharose sowie UDP-Glukose werden respiratorischer und glykolytischer Abbau von Glukose stark gesteigert, und die Schwellung der Schnitte nimmt um 50% ab.

J. DITTMANN[15]

Neurochirurgische Universitäts-Klinik, D-665 Homburg (Saar, German Federal Republic BRD), 17 August 1973.

[15] The technical assistance of D. GEMM, E. SEIFERT and H. VERFÜRTH is gratefully acknowledged.

Occurrence of Light-Dependent Acetylcholine Concentrations in Higher Plants

Although it is well established that acetylcholine (ACh) is widely distributed in the animal kingdom, only scanty information is available about the occurrence and function of ACh in the plant kingdom. Acetylcholine has been detected in a small number of plants by bioassay[1]. Although most biological test objects exhibit extreme sensitivity to ACh, bioassay procedures are susceptile to artifacts arising from unknown components of plant tissue extracts.

In a recent study, ACh has been identified in moss callus by a specific gas chromatographic method[2]. It has been found that the ACh content both in moss callus[2] and in secondary roots of mung beans[3] is regulated by phytochrome-mediated processes.

The present paper describes the determination of ACh with the gas chromatographic method in different plants which have become standard objects for the investigation of photomorphogenetic responses.

Materials and methods. The locally obtained seeds were grown in vermiculite in the green house. The etiolated seedlings were grown in the same substrate in a thermoconstant room in total darkness. The periods of cultivation differed depending on the plant species used (8–20 days). The dark grown plants were used for study 8 days after the germination. The plants were deep frozen and stored at −28°C. 20 g fresh weight of plant tissues were homogenized with a Bühler-homogenizer for 1 min with 10,000 rpm at +4°C. The extracting medium (50 ml) was 80% ethanol with 2% acetic acid (pH 3.9). The subsequent extraction procedure and the gas chromatographic estimation of ACh were performed as previously described[4,2]. The gas chromatographic analysis of all plant extracts yielded peaks whose retention times were identical with that of authentic ACh. In order to establish rigorously that the peaks from plant extracts were attributable to ACh, the extracts from each plant were first submitted to high voltage paper electrophoresis, and then analyzed by gas chromatography.

For electrophoresis the supernatant of the centrifuged plant extracts was concentrated by evaporation to 3 ml; 1.2 ml of each extract was submitted to electrophoresis (aliquots of 200 µl for each separation, paper 'Schleicher and Schüll 2043 Mgl', pyridin (0.3 *M*) formic acid

[1] V. P. WHITTAKER, *Handbuch der experimentellen Pharmakologie* (Springer, Berlin, Heidelberg, New York 1963), vol. 15, p. 1–39.
[2] E. HARTMANN and H. KILBINGER, Biochem. J. *137*, 249 (1974).
[3] M. J. JAFFE, Pl. Physiol. *46*, 768 (1970).
[4] H. KILBINGER, J. Neurochem. *21*, 421 (1973).

(0.66 *M*) buffer, pH 4.6, 1500 V, 1.5 h). The zone on the electrophoresis paper corresponding to authentic ACh was cut and eluted with 1 ml of 0.1 *M* NaH_2PO_4 buffer. Gas chromatography of the eluate yielded a peak with the same retention time as authentic ACh. These data provide evidence for the authenticity of the compound in the plants as ACh. No other choline ester among the lower homologues of ACh (propionyl-, butyryl-, valerylcholine) was found in these plants. Quantitative estimations were performed by gas chromatography without prior electrophoresis.

Results and discussion. ACh could be detected in all plants tested (Table). Thus it is possible that ACh occurs ubiquitously in the plant kingdom. The different plant organs, such as leaves, stem, roots showed various endogenous amounts of ACh, but the root system always contained less ACh than the shoot. As can be seen from the Table, ACh was not detected in etiolated seedlings. In another series of experiments, the effect of light intensity on the ACh concentration in peas (*Pisum sativum* L.) was investigated. After having grown in white light of an intensity of 7.5 J/m²/sec, these plants contained much less ACh (1.5 nmol/g) than peas that had grown under normal light conditions (31 J/m²/sec). In this particular experiment, the quantitative result obtained by gas chromatography was compared with that obtained by bioassay. The eluate from electrophoresis was assayed on the rat blood pressure[5]. There was no difference between the ACh concentration as estimated by gas chromatography or by bioassay (1.9 nmol/g). Our findings are in keeping with the previous observation that light has a regulatory effect on the ACh levels in plants[2].

JAFFE[3], YUNGHANS and JAFFE[6] suggested that ACh may act as a permeability regulator or as a local hormone, both in plants and in animals. The questions as to the site of synthesis and function of ACh in plants remain obscure[7–10]. The effect of light might be to increase the rate of synthesis of ACh, possibly by activating the enzymes responsible for biosynthesis.

The answer to this question depends on a better understanding of how biosynthesis of ACh occurs in plants. A basis for interpretation is information, essentially biochemical in nature, about the enzyme of synthesis, its properties and intracellular location and its functional behaviour in relation to other metabolic systems of the plant cell[11].

ACh content of higher plants

Plant	ACh (nmol/g fresh weight)
Phaseolus vulgaris	
stem	7.4 ± 1.7 (7)
leaves	2.0 ± 0.6 (7)
roots	0.4 (1)
Pisum sativum (light grown)	
shoot	8.2 ± 1.0 (10)
roots	1.4 ± 0.2 (3)
Pisum sativum (etiolated)	
shoot	nil (< 0.01) (2)
roots	nil (< 0.01) (2)
Sinapis alba (light grown)	
shoot	1.8 ± 0.4 (5)
Sinapis alba (etiolated)	
shoot	nil (< 0.01) (2)
Cucurbita pepo	
shoot	10.5 ± 0.9 (8)
roots	3.3 ± 0.3 (6)
Helianthus annuus	
shoot	7.9 ± 2.0 (9)
roots	3.5 (1)
Spinacea oleracea	
shoot	6.8 ± 0.7 (6)
Amaranthus caudatus	
shoot	3.1 ± 0.4 (4)

Results are mean ± SEM of the number of ACh determinations in parentheses. The lower limit of sensitivity of the method was about 0.10 nmol of ACh, which is equivalent to 0.01 nmol/g fresh weight of plant tissue.

Zusammenfassung. Der ACh-Gehalt verschiedener höherer Pflanzen wurde mit einer gaschromatographischen Methode bestimmt. Die Identifizierung des in den Pflanzen vorkommenden ACh erfolgte durch hochspannungselektrophoretische Auftrennung des pflanzlichen Extraktes und nachfolgender Gas-Chromatographie. Der Spross enthält mehr ACh als die Wurzel. In etiolierten Pflanzen war kein ACh nachweisbar. Es wird vermutet, dass der endogene ACh-Gehalt durch Licht reguliert wird.

E. HARTMANN and H. KILBINGER

Institut für Allgemeine Botanik der Universität, Saarstrasse 21, D-65 Mainz (German Federal Republic, BRD); and Pharmakologisches Institut der Universität, Obere Zahlbacherstrasse 67, D-65 Mainz (German Federal Republic, BRD), 19 August 1974.

[5] D. W. STRAUGHAN, J. Pharm. Pharmac. *10*, 783 (1958).
[6] H. YUNGHANS and M. J. JAFFE, Pl. Physiol. *49*, 1 (1972).
[7] E. HARTMANN, Z. Pflanzenphysiol. *71*, 349 (1974).
[8] H. KASEMIR and H. MOHR, Pl. Physiol. *49*, 453 (1972).
[9] R. L. SATTER, P. B. APPLEWHITE and A. W. GALSTON, Pl. Physiol. *50*, 523 (1972).
[10] T. TANADA, Pl. Physiol. *49*, 860 (1972).
[11] We wish to thank Mrs. U. KNÜPPEL and Miss A. MUTH for skilful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

Sur les relations entre la production d'hexosamines et d'hydroxyproline de la part de fibroblastes cultivés in vitro dans différentes concentrations d'O_2

Le collagène, associé aux mucopolysaccharides, constitue le composant le plus important de la substance intercellulaire et des différents types de tissus conjonctifs. Ces deux substances sont produites principalement par les fibroblastes.

Dans des travaux précédents, nous avons étudié la morphologie, l'activité enzymatique, le métabolisme du DNA et la production d'hexosamines de la part des fibroblastes cultivés in vitro en différentes concentrations d'O_2[1–4]. Il nous a paru intéressant d'étudier dans les