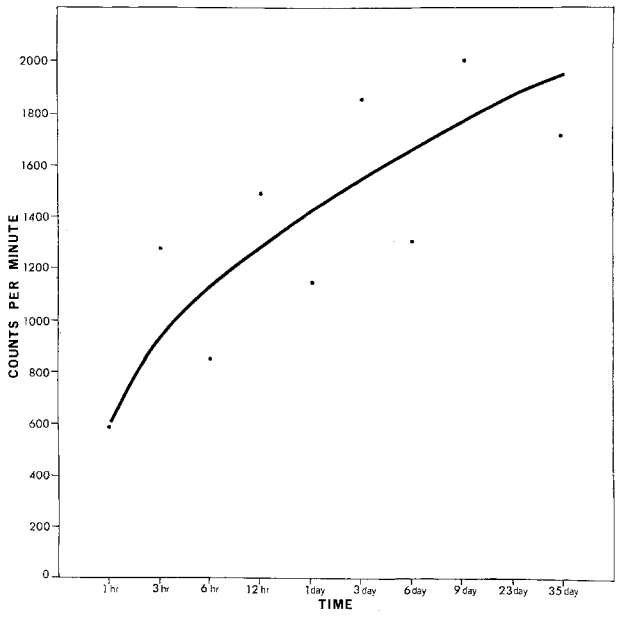

Fig. 2. ^{35}S recovery over time from the total residual pellet from heads of 5 insects, each insect initially injected with 124,231 cpm.

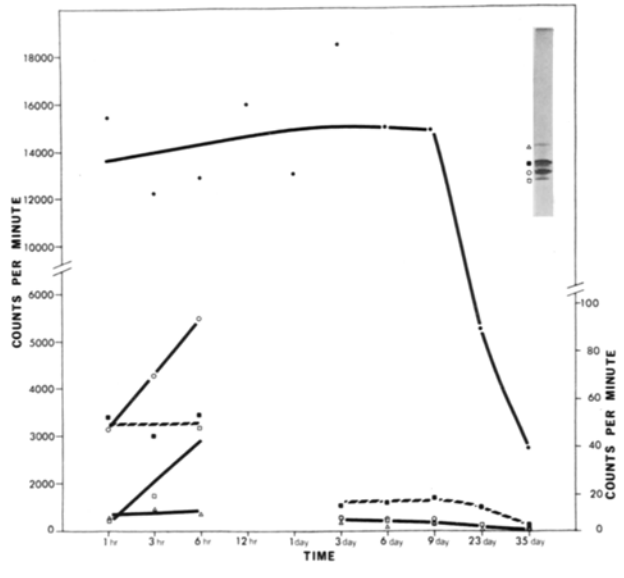

Fig. 3. Same as Figure 1 except involves the total saline-soluble fraction, and electrophoreted protein bands.

After 3 days, the greatest ^{35}S incorporation into soluble protein was in the band (Figure 3, solid black squares) which shows the electrophoretic characteristics of the membrane-associated chemoreceptor protein in the T_1 extract (Figure 1, solid black square).

Although ^{35}S incorporation into these protein fractions has been demonstrated, a fraction of the intitial rapid label uptake (1 to 24 h) may have been due to a formation of mixed disulfides. Reduction with excess thiol, column fractionation and electrophoresis of these ^{35}S incorporated extracts[12] demonstrated that the label recovered in the T_1 extract after 3 days of in vivo incubation was essentially all incorporated into the polypeptide chain of the receptor protein.

Rozental and Norris[10,11] have shown that messenger quinones cause characteristic conformational changes in the T_1 receptor protein, but not in S_1 proteins. The T_1 receptor protein is an integral component of the nerve membrane[7,9] and its subunits become characteristically aggregated or unfolded in response to specific messengers[10,11]. Though characteristic aggregation or unfolding in response to messengers has not been found with S_1 proteins[10,11], the S_1 proteins will bind messenger quinones under physiological conditions[12]. Our tentative interpretation is that the ^{35}S labeled S_1 proteins which are apparent precursors (subunits) of the membrane-associated receptor contain thiol groups and bind messenger, but that these saline-soluble proteins lack the abilities of the membrane-bound T_1 receptor to predictably change conformation in response to each messenger, as occurs in the energy-transferring mechanism in the sensory nerve membrane during chemoreception.

Resumen. (^{35}S) cistina injectada en la cavidad sanguínea abdominal fue incorporada en una proteína quemoreceptora aislada de tejido nervioso. La incorporación empezó durante la primera hora, llegó a una máximo a las 24 h, se mantuvo a este nivel por 9 días y a los 23 días había declinado.

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642 Russell Laboratories, University of Wisconsin, Madison (Wisconsin 53706, USA), 18 June 1974.

[12] G. Singer and D. M. Norris, unpublished data.

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Swelling of Brain Slices: Non-Osmotic Reversion Caused by Metabolic Intermediates

During in vitro incubation mammalian brain slices take up water[1]. The swelling increases in hypotonic solutions, in anoxia[2], after addition of inhibitors or uncouplers of the glucose metabolism[3], and finally in the presence of glutamate if the medium contains potassium[4]. A complete prevention of the swelling is not possible; but the water uptake is reduced, if the preparation of the slices is made under mild conditions[5], if efficient oxygenation is provided for[2], if chloride in the medium is substituted by isethionate[6], or if hypertonic solutions are used[7,8]. Okamoto and Quastel[9] observed almost

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Table I. Composition of mixture Q

Substance	Concentration (mM)
Succinic acid	0.5
Cytidine-5′-diphospho-choline	10
Fructose-1.6-diphosphate	15
Glycerate-3-phosphate	15
Lactose	10
Uridine-5′-diphospho-glucose	5

CDP-choline was used as the Na salt, UDP-glucose as the Na_2 salt, fructose diphosphate and glycerate phosphate as Na_3 compounds. For a neutralization of succinic acid and fructose diphosphate between 0.3 and 0.6 moles of Na_2CO_3 were added per 1 mole of fructose diphosphate, the acid content of the latter varying somewhat in the different lots of fabrication.

linear relationships between increase of intracellular ATP concentrations and decrease of the water content of rat brain slices. This paper gives a short report about my search for substances which diminish the water content of brain slices even when the osmotic pressure of the solutions is not enhanced.

Method. After killing rabbits by air embolism, their brains were removed and put into cold solution ('K-solution') of the composition (mM): NaCl 106, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.8, Na_2HPO_4 1.2, KH_2PO_4 0.3, $NaHCO_3$ 25, glucose 5. Cortex slices, about 0.5×0.5 mm in area and not more than 0.3 mm thick, were cut with a scalpel in the cold. O_2 uptake and CO_2 production were measured by WARBURG's two-vessel manometric method[10] under N_2 with 5% CO_2 and 8% or 40% O_2. 8% O_2 and 40% O_2 were chosen because these values were missing from earlier experiments[11]. They represent hypoxic and normal incubation conditions. The single vessels contained between 50 and 100 mg of tissue fresh weight. After incubation of 3 h, the slices were separated from the solutions by centrifugation for 10 min at $3000 \times g$. Adherent liquid was drained off, the wet weight of the slices and their weight after drying for 16 h at 105 °C were determined. The relative water content of another piece of brain cortex was determined in the same way without slicing and incubating. The production of lactate and pyruvate and the consumption of glucose were measured in the K-solution by enzymatic methods[12]. The compounds were dissolved in K-solution; in the beginning, these solutions were in the sidearms of the vessels; after measuring the metabolism for 1 h, the solutions of substances were added to the main compartments of the vessels, and the experiments were continued for a further 2 h.

Results and discussion. The controls had a swelling of 60–90% of the initial fresh weight, depending on the mode of incubation, e.g. the supply of O_2. The swelling was reduced by about 10% in the presence of (mM): Phosphate 5, $CaCl_2$ 5, fructose-1-phosphate 10, fructose-6-phosphate 10, fructose-1.6-diphosphate 5, glucose-1-phosphate 5, glucose-6-phosphate 10, glycerate-3-phosphate 5, lactose 10, sucrose 10, glycerol 50, ATP 2, CDP 4, cytidine-5′-diphospho-ethanolamine 4, cytidine-5′-diphospho-choline 4, UDP 2, uridine-5′-diphospho-glucose 5. The decreases of the water content were significant.

0.5 mM succinate had a small effect, smaller concentrations were ineffective, higher concentrations increased the water content by inhibition of the glycolysis analogous to iodoacetic acid and glyceraldehyde. The swelling is also enhanced by the uncoupler 2,4-dinitrophenol and by ethacrynic acid[13], escin, indomethacin and buformin[14].

After examining the effects of increasing the concentrations of substrates, singly or in combination, on metabolic changes or on water extrusion, I prepared a mixture of 6 substances called 'mixture Q' (Table I). At the present state of my experience this is a useful combination of some of the aforementioned substances. The combination is more efficient than a mere increase of the concentration of one substance to 30–50 mM. Succinate is a constituent of mixture Q, because it has hyperadditive effects on respiration and glycolysis if applied together with some of the other compounds. Lactose can be replaced by an equal concentration of sucrose. During various experiments, the changes of osmotic pressures caused by addition of substances or by omission of part of the NaCl were measured by freezing point determina-

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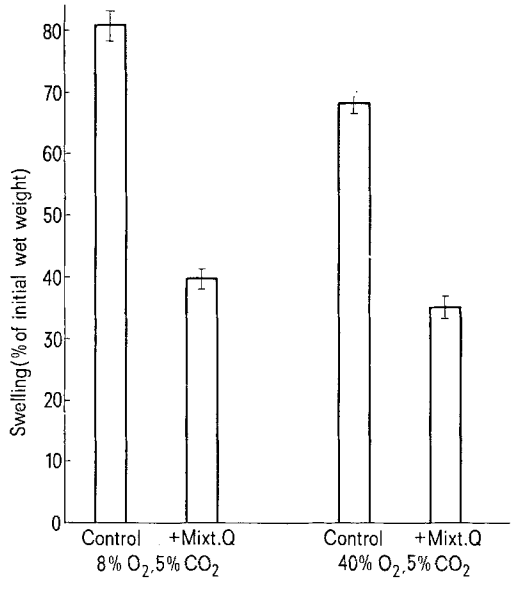
Table II. Influence of mixture Q on the glucose metabolism of brain cortex slices

Parameter	Control	+ Mixture Q
N_2 with 8% O_2 and 5% CO_2		
QO_2 ($\mu l \cdot mg^{-1} \cdot h^{-1}$)	$-$ 4.5 $\pm$ 0.1	$-$ 5.0 $\pm$ 0.1
QCO_2 ($\mu l \cdot mg^{-1} \cdot h^{-1}$)	7.3 $\pm$ 0.3	10.6 $\pm$ 0.3
Glucose consumption ($\mu g \cdot mg^{-1} \cdot h^{-1}$)	20.9 $\pm$ 0.9	38.6 $\pm$ 1.3
N_2 with 40% O_2 and 5% CO_2		
QO_2 ($\mu l \cdot mg^{-1} \cdot h^{-1}$)	$-$ 6.0 $\pm$ 0.1	$-$ 8.7 $\pm$ 0.2
QCO_2 ($\mu l \cdot mg^{-1} \cdot h^{-1}$)	8.0 $\pm$ 0.1	12.1 $\pm$ 0.2
Glucose consumption ($\mu g \cdot mg^{-1} \cdot h^{-1}$)	17.7 $\pm$ 0.9	31.3 $\pm$ 1.4

Mean values $\pm$ S.E.; $N = 8$ for each series of experiments. Glucose and oxygen consumption and carbon dioxide production are based on the final dry weight.

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.

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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

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