quency higher than the highest peristaltic rate recorded, instead of the reflex inhibitory mechanism which they proposed. However, the ureter has been examined from a feedback control systems point of view[9] and the existence of such a mechanism cannot be ruled out.

The pacemaker frequency in both guinea-pig and sheep was remarkably stable. The standard deviations of groups of 35 consecutive pacemaker potentials in the sheep ranged from 2% to 6% of the mean, so that the spread of the histograms is more likely to be due to variability of the threshold of excitation in the pyeloureter, which acts as a minor delay mechanism. The pyeloureter also acts as a gate, preventing most pacemaker potentials from initiating peristaltic waves.

Two possible influences on this proposed gating or filtering mechanism were investigated. Figures 4B and 4C show distributions obtained before and after 25% stretch was applied to the whole preparation. This allowed more contractions to occur at lower multiples of the pacemaker period, presumably by lowering the threshold of excitation in the pyeloureter. The action of different temperatures on the isolated guinea-pig ureter likewise changed the gate threshold. In the temperature range 29–33 °C, the distributions were unimodal with periods of 30 sec, but in the range 34–37 °C the distributions became multimodal, with peaks at approximately 30, 20 and 15 sec.

Thus, although the pacemaker is a necessary condition for the initiation of peristaltic waves, an additional stimulus in the pyreloureter, by way of distention due to accumulated urine in the renal pelvis, is required before the peristaltic wave can be triggered. Such a mechanism would effectively isolate the renal compartment hydrodynamically from the urinary bladder. During diuresis, however, the distension in the renal pelvis would lower the pyeloureteral gate excitation threshold in a manner similar to that demonstrated experimentally by the application of stretch as in Figure 4C. A full renal pelvis would therefore give rise to a unimodal distribution of peristaltic intervals, with a pacemaker to ureteral contraction ratio of 1:1. Under such conditions, hydrodynamic isolation of the kidney would still occur due to the continued maintenance of regular peristaltic contractions by the renal pacemaker.[10]

Summary. Ureteral contractions occur at intervals which are integral multiples of the period of pacemaker potentials recorded in vitro from the renal pelvis with a sucrose gap, suggesting that a gating mechanism in the pyeloureter regulates the rate at which the pacemaker initiates contractions.

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Department of Zoology, University of Melbourne, Clayton (Victoria 3168, Australia), 16 December 1974.

[9] R. Absher, in *Urodynamics: Hydrodynamics of the Ureter and the Renal Pelvis* (Eds. S. Boyarsky, C. W. Gottschalk, E. A. Tanagho and P. D. Zimskind, Academic Press, New York 1971), p. 233.

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Inhibition of Light-Induced, Transient Membrane Potential Oscillations of *Oenothera* Leaf Cells by Cycloheximide

The antibiotic cycloheximide (CH), known as a specific inhibitor of protein synthesis in 80 S (cytoplasmic) ribosomes of eucaryotic cells[1], has recently been used in studies of ion uptake and transport in plants[2–5]. Besides protein synthesis, CH inhibits transport of ions through barley roots, whereas ion accumulation from the ambient medium is not affected[3,4]. It was suggested that CH exerts its effect on symplasmic ion transport in the root parenchyma by interaction with the endoplasmic reticulum (ER) and its membrane turnover[3,4]. In the experiments described here, we attempted to investigate whether CH also affects other symplasmic phenomena such as electrical coupling between neighbouring cells in plant tissues.

For demonstration of electrical coupling, we used the internally generated signal of the well-known transients of electrical potential difference (PD) which are triggered by switching on or off photosynthetic energy transfer reactions (review[6]). These PD transients also occur in normally green cells of variegated leaves of *Oenothera*-mutants. PD transients are not observed, however, in the yellowish mutated cells having an impaired photosystem I or photosystem II respectively, unless there is a symplasmic connection between the green and the mutated cells[7]. This shows that the light-triggered signal can be translocated from the green cells to the mutated cells.

In our experiments, we used micro-capillary electrodes (tip diameter < 1 µm, filled with 3 *M* KCl, resistance > 4 Mohms in 3 *M* KCl). The electrodes were inserted into the center (probably the vacuole) of a cell of the upper palisade parenchyma layer, after the epidermis was stripped off. The leaf sample was mounted into a small chamber which was continuously flushed with artificial pond water (APW: 1 m*M* NaCl + 0.1 m*M* KCl + 0.05 m*M* $CaSO_4$, pH about 6.1). For more experimental details see[8].

Initial experiments showed that after addition of CH (10 µg/ml = 36 µ*M*) to the external medium, no light-induced potential changes were detectable with the electrode tip in white cells of *Oenothera hookeri · albicans* IV/IIα, although green cells were in symplasmic contact with the mutated cells. Thus, apparently CH blocked symplasmic transfer of the signal.

[1] H. D. Sisler and M. R. Siegel, in *Antibiotics* (Eds. D. Gottlieb and P. D. Shaw; Springer, Berlin-Heidelberg-New York 1967), vol. 1, p. 283.

[2] R. J. Ellis and I. R. MacDonald, Plant Physiol. *46*, 227 (1970).

[3] A. Läuchli, U. Lüttge and M. G. Pitman, Z. Naturforsch. *28c*, 431 (1973).

[4] U. Lüttge, A. Läuchli, E. Ball and M. G. Pitman, Experientia *30*, 470 (1974).

[5] R. Jeanjean, FEBS Lett. *32*, 149 (1973).

[6] F. W. Bentrup, Fortschr. Bot. *33*, 51 (1971).

[7] E. Brinckmann and U. Lüttge, Planta *119*, 47 (1974).

[8] E. Brinckmann, Dissertation T. H. Darmstadt, 1973.

Respiratory O_2-uptake, photosynthetic O_2-evolution, $^{14}CO_2$-fixation, and light-dependent pH-changes in the external medium of completely green leaf strips of *Oenothera hookeri · albicans* IV/IIα in the absence (controls) and the presence of 10 µg CH/ml after 1 h preincubation in the aerated solution

	Controls	10 µg CH/ml	n	P
O_2-uptake (µmole g_{DW}^{-1} h^{-1})	125 ± 18	101 ± 13	5	> 0.9
O_2-evolution (µmole g_{DW}^{-1} h^{-1})	304 ± 51	239 ± 29	5	> 0.9
$^{14}CO_2$-fixation (µmole g_{FW}^{-1} h^{-1})	125.6 ± 2.7	120.8 ± 11.8	4	> 0.6
Light-induced H^+-uptake (µmole g_{DW}^{-1} h^{-1})	111 ± 9	100 ± 6	5	> 0.3
Dark-induced H^+-release (µmole g_{DW}^{-1} h^{-1})	77 ± 8	78 ± 8	5	> 0.8

Deviations are standard errors of the mean, n is the number of replicates, P was calculated by t-test after Student, FW, fresh weight; DW, dry weight.

However, 2 control experiments with the electrode tip in fully green leaf cells of this variegated mutant showed that the PD oscillations in the green cells themselves are inhibited by CH. Figure 1 shows one of these experiments: in the first 22 min, normal transient changes are exhibited after switching on or off the microscopic light. After 22 min, the external medium was replaced by the CH-containing solution. Now the electrode was consecutively inserted into 2 new cells of the same green leaf sample as indicated in Figure 1 which shows that due to the addition of CH the PD oscillations disappeared with time, except for small and rather short distortions of the PD from the resting level. In wild-type *Oenothera*-leaf cells under normal experimental conditions, large transient oscillations are detectable even 100 min after insertion of an electrode[8]. Figure 2 depicts the CH-dependent decrease of the amplitude of the major light-triggered depolarization.

Since light-dependent PD changes are linked to photosynthetic electron flow[6-12], we had to ensure that photosynthesis itself was unaffected by CH. Therefore, we measured photosynthetic O_2-production, $^{14}CO_2$-fixation, light-induced pH-changes in the external medium, and in addition respiratory O_2-consumption. The leaf strips (about 1 cm long and 1 mm wide) were preincubated for 1 h in the experimental solution (APW with 10 µg CH/ml added where appropriate). O_2-exchange of leaf strips was then recorded polarographically. $^{14}CO_2$-fixation (1 min) was determined under the same light- and temperature-conditions (10000 lux, 25 °C, pH 6.0). Light-induced pH-changes were measured as described previously[13]. These pH-changes (possibly brought about by apparent proton fluxes[9,10,13-15] as given in the Table) are dependent on intact non-cyclic photosynthetic electron flow[9,10,13-15]. They are kinetically correlated with the light-induced membrane potential changes in plant cells[9,10].

The Table shows that CH has no significant effect on the parameters tested, whereas photosynthesis-dependent PD oscillations are prevented by CH under the same conditions. In agreement with[2], CH had no effect on the respiration of the leaf strips.

These results suggest that CH blocks the communication between the processes within the chloroplasts generating the PD oscillations and the tip of the electrode picking up these transients. Having no effect on photosynthesis itself, CH probably does not affect the reactions triggering the signal. Since in our experiments the electrode tip was presumably in the vacuole, communication between the interior of the chloroplasts and the electrode extends over several membranes (e.g. thylakoid membranes, chloroplast envelope, tonoplast) and the cytoplasmic phase. CH may

[9] C. K. PALLAGHY and U. LÜTTGE, Z. Pflanzenphysiol. *62*, 417 (1970).
[10] V. K. ANDRIANOV, A. A. BULYCHEV, G. A. KURELLA and F. F. LITVIN, Biofizika *16*, 1031 (1971).
[11] G. THROM, Z. Pflanzenphysiol. *65*, 389 (1971).
[12] W. J. VREDENBERG and W. J. M. TONK, Biochim. biophys. Acta *298*, 354 (1973).
[13] A. B. HOPE, U. LÜTTGE and E. BALL, Z. Pflanzenphysiol. *68*, 73 (1972).
[14] E. BRINCKMANN and U. LÜTTGE, Z. Naturforsch. *27b*, 277 (1972).
[15] U. LÜTTGE, D. KRAMER and E. BALL, Z. Pflanzenphysiol. *71*, 6 (1974).

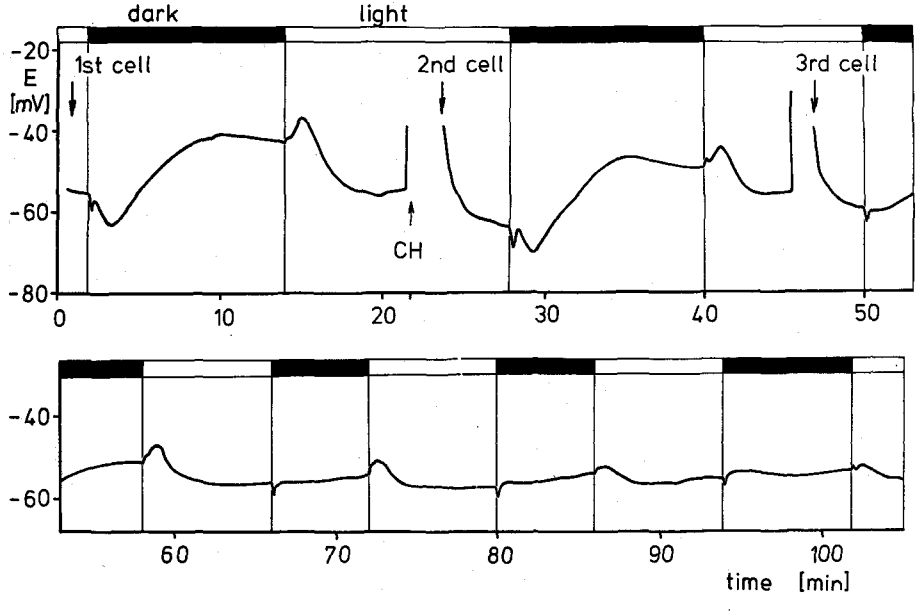

Fig. 1. Light- or dark-induced membrane potential oscillations. Electrical potential difference (E) between the inside of the cell (negative) and the experimental solution was recorded with the electrode tip inserted consecutively into 3 palisade parenchyma cells of a completely green leaf sample of *Oenothera hookeri · albicans* IV/IIα. At time zero, the electrode was inserted into the first cell and 2 transient potential changes were recorded with this cell in the absence of CH. After 22 min CH (10 µg/ml) was added to the continuously flowing external solution, and the electrode inserted into another cell. From here on, the recording shows the gradual dissappearing of the transient oscillations. The electrode was inserted into a 3rd cell after ~45 min. The signal has almost disappeared after about 60 min after CH addition.

affect this communication by acting on the movement of transport metabolites carrying ~ P and reducing equivalents across the chloroplast envelope[11,12]. Though we did not measure it in the present study, it is likely that CH inhibits protein synthesis in *Oenothera* leaf cells. Thus CH might cause the observed effect on PD oscillations via affecting processes with high rates of protein turnover as discussed in[4,16,17]. The relationship between proteins and the rather rapid changes of the membrane potential is, however, difficult to understand. It may be possible that electrogenic pumps in the membrane having a proteinaceous component are affected. But before we know all effects of CH on physiological processes in the cell, our conclusions necessarily remain more or less speculative.

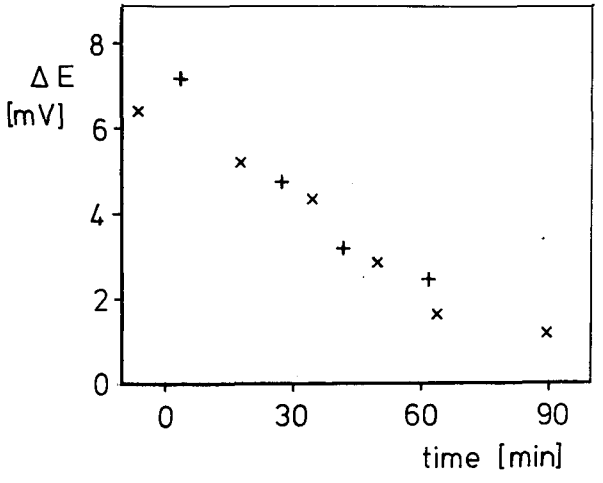

Fig. 2. Effect of CH on the amplitude (△ E) of the major light-induced depolarization of the membrane potential of *Oenothera* leaf cells from the same plant as in Figure 1. At time zero, CH was added (10 µg/ml). Results of 2 experiments (× and +) are shown; data given by ×-symbols have been taken from Figure 1.

Summary. The antibiotic cycloheximide (10 µg/ml) inhibits the light-induced transients of membrane potential of green cells in *Oenothera*-leaves, while photosynthesis (measured by O_2-evolution, $^{14}CO_2$-fixation and light-induced pH-changes in the external medium) and respiration remain unaffected under the same conditions.

Zusammenfassung. Unter dem Einfluss von Cycloheximid (10 µg/ml) werden die lichtausgelösten Membranpotentialschwankungen in grünen Mesophyllzellen von *Oenothera* unterdrückt, während unter den gleichen Bedingungen nach einer Stunde Vorbehandlung mit Cycloheximid Reaktionen der Photosynthese und der Atmung (gemessen als O_2-Entwicklung, $^{14}CO_2$-Fixierung, lichtinduzierte pH-Änderungen im Aussenmedium und als respiratorische O_2-Aufnahme) nicht beeinträchtigt werden.

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17 February 1975.

[16] E. Marrè, P. Lado, A. Ferroni and A. Ballarin Denti, Plant Sci. Lett. *2*, 257 (1974).
[17] R. Cleland, Planta *99*, 1 (1971).
[18] Permanent address: Botanisches Institut, Mittlerer Dallenberweg 64, D–8700 Würzburg, BRD.
[19] We thank Prof. Dr. W. Stubbe (Düsseldorf) for his gift of the *Oenothera* plants and Fräulein E. Ball for performing the $^{14}CO_2$-fixation experiments. This investigation was supported by a grant from the Deutsche Forschungsgemeinschaft.

Energetics of Locomotion in a Monotreme, the Echidna *Tachyglossus aculeatus*

The echidna *Tachyglossus aculeatus* appears uniquely useful as an analogue for studies of the energetic requirements of locomotion. The animal presents three features which we postulate may have an effect on the cost of locomotion; a resting oxygen consumption about one-half that found in placental mammals of the same body mass[1], a unique locomotory movement which involves humoral long-axis rotation rather than anteroposterior protraction[2] and distally heavy limbs specialized for digging. We report here experimental determinations of oxygen consumption during walking in the echidna and the relevance of these features to locomotory energetics.

The animals were taken from Kangaroo Island, South Australia, and maintained in the laboratory on artificial diet. From an initial sample of 4, 2 of the echidnas were trained to walk at a variety of speeds on a motor-driven treadmill. The ability of the animals to retract the head made the collection of respiratory gas in a mask impracticable, and all tests were conducted in a closed chamber with a port for incoming air at the rear. Steady state oxygen consumption was measured by drawing room air through the chamber at 16 l min^{-1} and measuring the difference in oxygen concentration of the gas from the chamber and that in room air by passing samples through a diaferometer[3]. Resting measurements were taken both before and after test runs on animals standing in the chamber under the same conditions of temperature and illumination. The animal was considered in steady state during activity since a) oxygen consumption varied less than ±3% over 15 min intervals, b) no increase in oxygen consumption was apparent in the rest period at the conclusion of the walking test. Ambient temperature in the walking chamber was maintained at 23 °C and all gas volumes are expressed as dry gas at standard conditions.

Steady state oxygen consumption increased linearly with running speed in both animals. For the larger (3.53 kg) echidna this relationship is described by the equation $M = 0.37\ V + 0.25$ and for the smaller (1.69 kg) animal by $M = 0.45\ V + 0.31$; where M is oxygen consumption in ml $O_2\ g^{-1}\ h^{-1}$ and V the walking velocity

[1] K. Schmidt-Nielsen, T. J. Dawson and E. C. Crawford jr., J. cell. Physiol. *67*, 63 (1966).
[2] F. A. Jenkins, Science *168*, 1473 (1970).
[3] We used a Kipp and Zonen model mg4 diaferometer coupled to a Kipp and Zonen micrograph model BD5. The unit was calibrated with a gas mixture at temperature and pressure conditions identical to the sample line.
[4] C. R. Taylor, K. Schmidt-Nielsen and J. L. Raab, Am. J. Physiol. *219*, 1104 (1970).