

affect this communication by acting on the movement of transport metabolites carrying $\sim P$ 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.

Summary. The antibiotic cycloheximide (10 $\mu\text{g/ml}$) inhibits the light-induced transients of membrane potential of green cells in *Oenothera*-leaves, while photosynthesis (measured by O_2 -evolution, $^{14}\text{CO}_2$ -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 $\mu\text{g/ml}$) 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, $^{14}\text{CO}_2$ -Fixierung, licht-induzierte pH-Änderungen im Aussenmedium und als respiratorische O_2 -Aufnahme) nicht beeinträchtigt werden.

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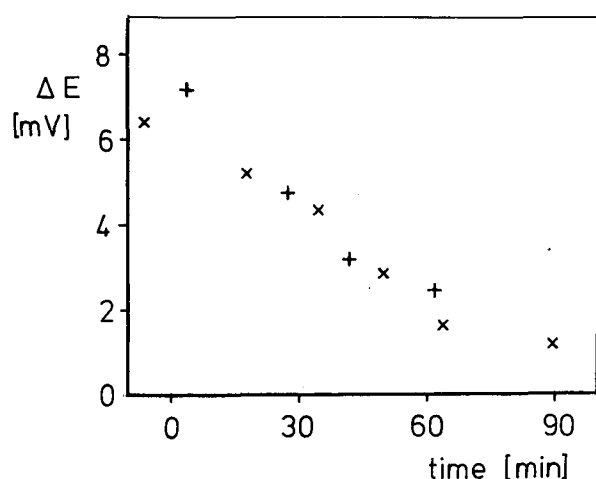


Fig. 2. Effect of CH on the amplitude (ΔE) of the major light-induced depolarization of the membrane potential of *Oenothera* leaf cells from the same plant as in Figure 1. At time zero, CH was added (10 $\mu\text{g/ml}$). Results of 2 experiments (\times and $+$) are shown; data given by \times -symbols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

¹⁶ E. MARRÈ, P. LADO, A. FERRONI and A. BALLARIN DENTI, *Plant Sci. Lett.* 2, 257 (1974).

¹⁷ R. CLELAND, *Planta* 99, 1 (1971).

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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 mass¹, a unique locomotory movement which involves humoral long-axis rotation rather than anteroposterior protraction² 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 diaferometer³. 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 $\pm 3\%$ 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 $\text{O}_2 \text{ g}^{-1} \text{ h}^{-1}$ 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).

in $Km\ h^{-1}$. The correlation co-efficients (r) for these least-square regressions were 0.96 and 0.88 respectively.

The gradient of such lines can be used to compare the cost-effectiveness of locomotion among different species. From the equations of TAYLOR, SCHMIDT-NIELSEN and RAAB⁴ the predicted gradient for a eutherian of equal body mass to the larger echidna is 0.32 and for the smaller, 0.43. We suggest that the rate of increase in steady state oxygen consumption with increasing walking speed in the echidna is similar to that found in other mammals.

The total oxygen consumed per unit body mass while travelling a given distance (defined as the cost of transport⁵, for the echidna is however lower than the predicted value⁴. Hence the change in power with change in velocity in the echidna is similar to that in eutherians, but the total energy requirement to travel the same distance is less. This is due to low resting oxygen consumption, the value of M when V is zero in the above equation. These extrapolated values are slightly above values previously determined for basal levels¹, but are still less than 60% of expected levels for eutherians⁶.

Most of the energy requirements for locomotion on level surfaces is believed to be dissipated as work in accelerating and decelerating limbs and in overcoming frictional resistance inherent in joints. As a corollary to the former it may be argued that a concentration of muscle mass close to the girdle insertion and an increase in the effective length of the limb (a progression towards the unguligrade condition) leads to a reduction in the moment of inertia of the limb and a corresponding decrease in locomotory energy requirements. The mammals whose running performances are used in scaling equations⁴ include some whose limb structure would support this view. The echidna however uses burrowing for both food gathering and evasion, and has short distally heavy limbs with pronounced elongation of the manus and pes. Despite this variation in limb configuration however, the rate of increase of oxygen consumption with velocity in the echidna is similar to that for other mammals.

Since locomotory costs at the speeds used in this experiment are therefore not apparently influenced by a distally massive limb or a unique form of humoral movement, we suggest three possible conclusions. Firstly, it is possible that the effect of these features is too small to observe as oxygen consumption. Alternatively, if a large

proportion of the energy invested in each limb movement is stored in elastic structures and returned during the next phase of the step cycle, the large moment of inertia of the limb is of little consequence. Finally, the proportion of energy expended in acceleration of the limbs may be very small.

From this consideration of echidna locomotion we have confirmed findings in other species. Work on the insectivore (*Tenrec ecaudatus*)⁷ and a variety of lizard species⁸ has previously suggested that rate of increase in power with speed is independent of gait, stance and resting metabolic levels. In another series of experiments⁹ involving mammals of similar body mass but with varying moments of inertia of limbs, the cost of locomotion was the same over a wide range of speeds. We have confirmed both of these findings in one species, the echidna. We suggest that it is possible to use scaling equations to predict oxygen demands in locomotion in animals of vastly different physiological and anatomical characteristics, however the reasons for the validity of these equations is still largely unknown.

Summary. The steady state oxygen consumption of two echidnas was measured during locomotion on a treadmill. The change in power input with change in velocity is similar to that found in other mammals, but the total energy requirement for locomotion is less. The significance of these findings in an animal with low basal metabolism and distally heavy limbs is discussed.

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⁵ K. SCHMIDT-NIELSEN, *Science* 177, 222 (1972).

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⁷ J. A. JAGGER, C. R. TAYLOR and A. W. COMPTON, *Fed. Proc.* 33, 349 (1974).

⁸ R. T. BAKKER, *Physiologist* 15, 76 (1972).

⁹ C. R. TAYLOR, A. SHKOLNIK, R. DMI'EL and D. BAHARAV, *Am. J. Physiol.* 227, 848 (1974).

¹⁰ Dr. BAUDINETTE's research is supported by the Nuffield Foundation.

Absence of Effects of Dibutyryl Cyclic Guanosine 3',5'-Monophosphate on Release of α -Amylase, ^{45}Ca Efflux, and Protein Synthesis in Rat Pancreas in vitro¹

In the exocrine pancreas, cholinergic agents stimulate both the release of α -amylase²⁻⁵, and the efflux of ^{45}Ca ⁶⁻⁸, while at the same time depressing protein synthesis⁹⁻¹¹. In some organs, cholinergic effects have been associated with a stimulation of the intracellular accumulation of cyclic guanosine 3',5'-monophosphate (GMP); and the emerging belief appears to be that this nucleotide mediates cholinergic action¹². The current study was designed to ascertain whether the dibutyryl analogue of cyclic GMP could mimic the carbachol effects on enzyme release, ^{45}Ca efflux and protein synthesis in the rat pancreas.

Materials and methods. Female Sprague-Dawley rats (180-225 g) were decapitated; excised pancreata were trimmed of adherent fat and mesentary and cut into fragments in chilled, oxygenated Krebs-Ringer bicarbonate buffer (Ca^{2+} adjusted to 0.05 mM). Secretion of

α -amylase (30 min stimulatory period) and efflux of ^{45}Ca were studied according to previously described techniques⁸.

Protein synthesis was measured by studying the incorporation of 3H -leucine into trichloroacetic acid-precipitable protein. Pancreatic fragments (~100 mg) were preincubated for 10 min in 2 ml buffer containing the various test agents. Two μ mole 3H -leucine (specific activity 0.5 μ Ci/ μ mole) were added and incubation allowed to continue for an additional 20 min. The reaction was stopped by homogenizing the tissue in its own medium, removing 50 μ l aliquots for protein determination¹³, and precipitating the remaining homogenate with an equal volume of cold 10% trichloroacetic acid. The acidified homogenate was centrifuged and the pellet washed 3 times with 5% trichloroacetic acid before being dissolved in 2 ml of formic acid. Radioactivity was subse-