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 RAAB[4] 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 transport:[5], for the echidna is however lower than the predicted value[4]. 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 levels[1], but are still less than 60% of expected levels for eutherians[6].

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 ungulligrade 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 equations[4] 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 species[8] has previously suggested that rate of increase in power with speed is independent of gait, stance and resting metabolic levels. In another series of experiments[9] 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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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 vitro[1]

In the exocrine pancreas, cholinergic agents stimulate both the release of α-amylase[2–5], and the efflux of ^{45}Ca[6–8], while at the same time depressing protein synthesis[9–11]. 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 action[12]. 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 techniques[8].

Protein synthesis was measured by studying the incorporation of ^{3}H-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 μmole ^{3}H-leucine (specific activity 0.5 μCi/μmole) 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 μl aliquots for protein determination[13], 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 disolved in 2 ml of formic acid. Radioactivity was subse-

quently measured in a 200 µl aliquot of the suspension, counts being corrected for background and efficiency.

Results. The dibutyryl analogue of cyclic GMP (10^{-7}–10^{-3} *M*) did not alter basal α-amylase release during a 30 min incubation period; cyclic GMP (10^{-3} *M*) itself was similarly ineffective even after 100 min of incubation (data not shown). As described in Table I, the analogue (10^{-3} *M*) also did not influence the secretory response to 10^{-5} *M* carbachol.

With respect to ^{45}Ca efflux, dibutyryl cyclic GMP (10^{-5} *M*) moderately increased the efflux rate, though non-significantly in comparison to the controls (Table II). Carbachol (10^{-5} *M*), on the other hand, increased the rate of release of the ion significantly, in comparison to untreated tissues, and at 5 and 15 min in comparison to tissues treated with dibutyryl cyclic GMP. The nucleotide did not affect the response to carbachol.

Whereas carbachol (10^{-5} *M*) depressed the rate of incorporation of ^{3}H-leucine into newly synthesized protein (Table III), this effect was not duplicated by dibutyryl cyclic GMP (10^{-5} *M*). In addition, the nucleotide did not alter the carbachol effect.

Discussion. In many cholinergically-innervated tissues, the effect of the neurotransmitter is associated with an intracellular accumulation of cyclic GMP[12]. To our knowledge, a similar correlation has not been reported in pancreas, though indirect evidence suggests that cyclic GMP may play a role in secretion of enzyme[14] or de novo protein synthesis[11] in this organ. Dibutyryl cyclic GMP has been used in other studies to mimic the muscarinic effects of cholinergics[15–18]. In the present study the nucleotide was biologically inert, perhaps as a result of the impermeability of rat pancreatic membranes to it. While not being sufficiently conclusive to eliminate cyclic GMP as an intracellular pancreatic mediator, the current findings do underline the fact that exogenous dibutyryl cyclic GMP may, in certain tissues, be an agent of limited usefulness in confirming the hormonal actions of parasympathomimetic substances.

Summary. Dibutyryl cyclic GMP did not affect basal, or carbachol stimulated secretion of α-amylase from rat pancreas. The nucleotide did not have a significant effect

Table I. Effects of dibutyryl cyclic GMP (10^{-3} *M*) on basal and carbachol (10^{-5}) *M* stimulated secretion of α-amylase from rat pancreas incubated for 30 min at 37 °C

Addition	Release (%)
None	5.87 ± 0.30
Dibutyryl cyclic GMP	6.85 ± 0.30
Carbachol	12.48 ± 0.22[a]
Dibutyryl cyclic GMP + carbachol	13.83 ± 0.61[a]

Values are means ± SEM of 4 observations. [a] $P < 0.001$.

Table II. Efflux of ^{45}Ca from rat pancreas incubated at 37 °C in the presence of dibutyryl cyclic GMP (10^{-5} *M*) or carbachol (10^{-5} *M*) or the two agents together

Addition	^{45}Ca remaining in Tissue (%)				
Incubation (min) [a]	5	10	15	20	30
None	70.3 ± 5.0	61.8 ± 4.9	49.0 ± 3.4	48.3 ± 3.3	46.2 ± 5.0
Dibutyryl cyclic GMP	59.6 ± 3.7	52.0 ± 5.2	47.8 ± 3.1	41.6 ± 2.3	34.7 ± 2.4
Carbachol	49.3 ± 1.9[b]	42.2 ± 3.9[d]	38.1 ± 2.4[c]	34.1 ± 3.0[d]	29.6 ± 3.2[d]
Dibutyryl cyclic GMP + carbachol	52.0 ± 3.5[c]	42.7 ± 4.9[c]	33.3 ± 1.9[c]	29.8 ± 3.7[c]	29.9 ± 2.8[c]

Values are means ± SEM of 4–5 observations. [a] Efflux is expressed as a percentage of the specific activity (dpm ^{45}Ca/mg protein) with respect to the specific activity in the 'Zero time' (unincubated) samples = 100%. [b] $p < 0.005$. [c] $p < 0.01$. [d] $p < 0.02$. [e] $p < 0.05$.

Table III. Effects of dibutyryl cyclic GMP (10^{-5} *M*) and carbachol (10^{-5} *M*) on protein synthesis in rat pancreas as measured by the incorporation of ^{3}H-leucine into trichloroacetic-acid insoluble protein

Addition	^{3}H-Leucine incorporated (dpm/mg/20 min)
None	879
Dibutyryl cyclic GMP	931
Carbachol	480
Dibutyryl cyclic GMP + carbachol	554

Observations repeated in quadruplicate; results presented are from a typical experiment.

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on ^{45}Ca release from the pancreas nor did it alter the response to carbachol. The dibutyryl analogue of cyclic GMP did not duplicate or alter the inhibitory effect of carbachol on ^{3}H-leucine incorporation into pancreatic trichloroacetic acid-precipitable protein.

Résumé. L'effet du dibutyryl GMPc sur la sécrétion d'α-amylase, l'efflux du ^{45}Ca et la synthèse de nouvelles protéines dans le pancréas du rat, ont été étudiés. Le dibutyryl GMPc n'affecte ni la sécrétion d'enzyme basale ni celle stimulée par le carbachol. Le nucléotide n'a pas un effet significatif sur le relâchement du ^{45}Ca du pancréas et n'altère pas la réponse du carbachol. L'analogue ne mime pas et ne change pas l'effet du carbachol sur l'incorporation du ^{3}H-leucine dans les protéines précipitées par l'acide trichloroacétique.

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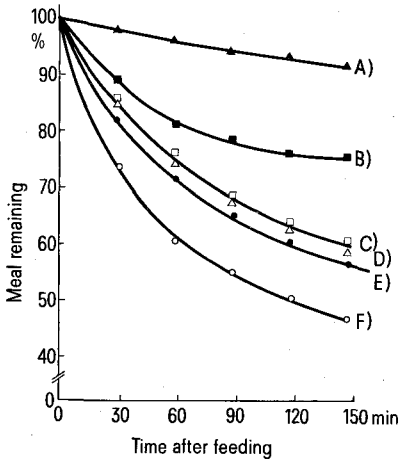
Inhibition of Diuresis in the Tsetse Fly (*Glossina morsitans*) by Ouabain and Acetazolamide

Recently TOBE[1] reviewed the literature dealing with diuresis following a blood meal in tsetse flies and presented evidence concerning water movements in female *Glossina austeni*. TOBE showed that $^{3}H_2O$ was rapidly eliminated after it had been ingested in a meal of defibrinated beef blood and that the specific activity in the urine was approximately the same as the specific activity in the meal; the specific activity of tsetse haemolymph was, however, considerably lower than the meal on which the flies fed. By feeding ^{14}C-carboxyl dextran in defibrinated beef blood, TOBE showed that 13 of 31 flies excreted significant amounts of ^{14}C within 5 min of feeding, but no ^{14}C was found in the haemolymph. TOBE proposed 2 models to explain the results: in the first model water passes directly down the gut and in the second model water passes through the gut wall and Malpighian tubules and there is a compartmentalization of the tsetse haemolymph. The evidence supported both models and it was not possible to choose between them. I therefore wish to report my observation that both acetazolamide and ouabain, metabolic inhibitors which interfere with certain membrane transport systems, reduce the rate of water elimination by male *Glossina morsitans morsitans*.

Materials and methods. G. morsitans used in this experiment were from our colony which is maintained by feeding on rabbits 6 days per week[2]. Previously unfed males were individually weighed (to 0.01 mg) when 24 to 48 h old, fed the experimental meal through an Agar/Parafilm membrane[3], held at ca. 22 °C and reweighed at 30 min intervals. The meal consisted of 0.85% NaCl, 1 m*M* ATP (adjusted to pH 7.4) with or without ouabain or acetazolamide.

Results and discussion. The 62 flies used in this experiment weighed an average of 16.41 ± 2.43 mg and consumed meals averaging 24.87 ± 5.34 mg. There was no significant difference between the meal size of flies fed upon saline and those fed upon saline plus ouabain or acetazolamide. The data in the Figure show a rapid loss of weight in *G. morsitans* males fed upon a saline solution. The excretion rate of the controls is comparable to the rate reported for *G. morsitans* males fed heparinized goat blood or haemolyzed bovine blood[4]. Both of the metabolic inhibitors ouabain and acetazolamide reduce the rate of water loss. Ouabain is an inhibitor which, in most animals, interferes with a Na^+/K^+ active transport system across membranes. Acetazolamide is a specific inhibitor of carbonic anhydrase and as such also interferes with ion and water transport across membranes[5]. Evidence for ouabain sensitive transport mechanisms in insects has been obtained with *Periplaneta americana*[6] and *Drosophila hydei*[7,8] but not *Rhodnius prolixus*[9] or *Carausius morosus*[10]. On the basis of the effects of Na^+ and K^+ upon water excretion LANGLEY and PIMLEY[4] suggested that an active transport system exists in *G. morsitans*. Carbonic anhydrase has been demonstrated in several tissues of a number



Effect of acetazolamide and ouabain on diuresis by male *G. morsitans* after feeding on a meal of 0.85% NaCl, 1 m*M* ATP at pH 7.4. Each point is the mean of 8 to 12 determinations; the number of flies used is given in parentheses. The standard deviation for each of the means was approximately 10% of that mean.
A) 300 μg/ml acetazolamide (12); B) 20 μg/ml ouabain (10); C) 0.2 μg/ml ouabain (8); D) 50 μg/ml acetazolamide (11); E) 0.2 μg/ml ouabain plus 50 μg/ml acetazolamide (11); F) control (10).

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