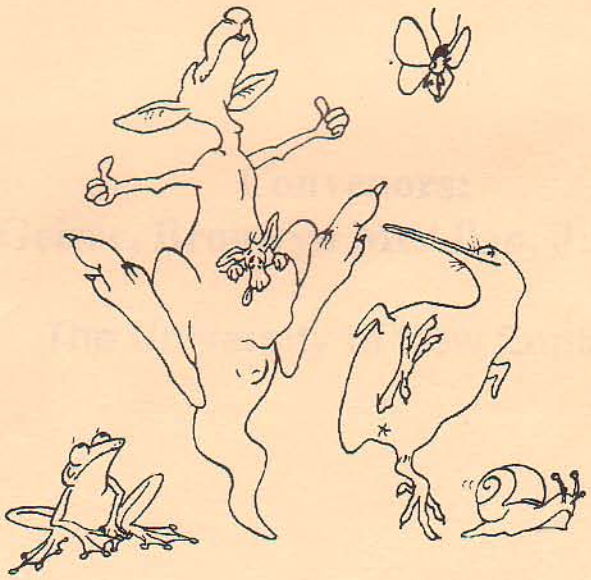
ANZSCPB
Proceedings

Australian and New Zealand Society for Comparative Physiology and Biochemistry

16th Annual Meeting
December 2 to 5 1999

The University of New England, Armidale



Programme & Abstracts

Sponsored by: The University of New England, Journal of Comparative Physiology/Springer, Comparative Biochemistry & Physiology/Permagon

ANZSCPB
Proceedings Volume 16

Australian and New Zealand Society for Comparative Physiology and Biochemistry

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Programme & Abstracts

Convenors:
Fritz Geiser, Bronwyn McAllan, Julie Roberts

The University of New England

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PROGRAMME

THURSDAY 2ND DECEMBER

4.00 PM - 6.00 PM
REGISTRATION, DRINKS & NIBBLES IN BIOLOGICAL SCIENCES FOYER

FRIDAY 3RD DECEMBER 1999

8.30 - 9.00	Registration in Biological Sciences Foyer
9.00 -9.10	Welcome
	Session One - Thermoregulation Chair: Stewart Nicol
9.10 - 9.30	The role of fur: polar bears versus koala 'bears'. T. J. Dawson*, S. K. Maloney and Koa Webster
9.30 - 9.50	Daily torpor and its energetic consequences in a small malagasy primate, *Microcebus murinus*. Jutta Schmid* and John R. Speakman
9.50 - 10.10	Torpor in free-ranging owlet-nightjars, *Aegotheles cristatus*. Fritz Geiser*, Gerhard Körtner, Tracy A. Maddocks, & R. Mark Brigham
10.10 - 10.30	Effects of a helium/oxygen atmosphere on thermoenergetics in the sugar glider, *Petaurus breviceps*. Joanne C. Holloway* & Fritz Geiser
10.30 - 11.00	*Morning tea*
	Session Two - Pulmonary physiology Chair: Harry Taylor
11.00 - 11.20	Prenatal development of the antioxidant enzyme system in the lungs of non-mammalian vertebrates. Adam Starrs, Olga Lopatko, Sandra Orgeig & Christopher B. Daniels*
11.20 - 11.40	Dexamethasone and adrenaline stimulate pulmonary surfactant secretion in embryonic chicken lungs. Lucy C. Sullivan*, Sandra Orgeig, and Christopher B. Daniels
11.40 - 12.00	Development of the pulmonary surfactant system in the snapping turtle, *Chelydra serpentina*. Sonya D. Johnston[1*], Susan Wert, Gary C. Packard, Sandra Orgeig, David Cenzato, Jeffrey A. Whitsett and Christopher B. Daniels.
12.00 - 12.20	Thermal and neural control of surfactant secretion from type II cells of the fat-tailed dunnart, *Sminthopsis crassicaudata*. Carol J. Ormond, Sandra Orgeig* and Christopher B. Daniels
12.20 - 12.40	Diurnal fluctuations in the pulmonary surfactant system in two species of bat. Nicola C. Slocombe, Jonathan R. Codd*, Philip G. Wood, Sandra Orgeig, and Christopher B. Daniels.
12.40 - 14.00	*Lunch*

FRIDAY 3RD DECEMBER 1999

13.30–15.10	**Session Three - Cardiovascular Physiology/Gas exchange** **Chair: Russell Baudinette**
14.00 - 14.20	Ventricular functioning in the bi-pyramidal heart of the eel. D. H. Rohr*, & P. S. Davie
14.20 - 14.40	Independent effects of gravity on cranial and caudal blood pressures in aquatic and terrestrial snakes Roger S. Seymour* and Joachim O. Arndt
14.40 - 15.00	The ventilatory response to CO_2 in reptiles. S. L. Munns* and B. K. Evans
15.00 - 15.20	Gas exchange in marsupial neonates: the role of the skin. Peter MacFarlane and Peter Frappell*
15.20 - 15.40	Cloacal ventilation and dive performance of the Fitzroy River turtle, *Rheodytes leukops* Craig E. Franklin and Toni Priest*
15.40 - 16.10	*Afternoon tea*
15.10 15.40	**Session Four - Energetics Chair: Terry Dawson**
16.10 - 16.30	The energetics of locomotion in vertebrates: After twenty years, where are the generalisations? R. V. Baudinette
16.30 - 16.50	The influence of age, organ mass, and testosterone on aerobic performance in house sparrows. W. A. Buttemer*, C. Bech, M. A. Chappell, and L. B. Astheimer
16.50 - 17.10	Locomotion energetics of the brush-tailed Bettong. Koa Webster* and Terence J. Dawson
17.10 - 16.40	**Poster session in Biological Sciences seminar room**
POSTERS	Faecal testosterone concentrations may not be useful for monitoring reproductive cycles in blue-tongued lizards. Natalia Atkins, Ashley Edwards* and Susan M. Jones.
	Crural muscle function in the pelvic limb of the turkey, *Meleagris gallapavo*. R. B. Campbell* and M. B. Bennett
	An alternative to 17β-oestradiol in a viviparous reptile? Ashley Edwards* and Susan M. Jones
	Effect of thermoperiodic acclimation on rhythms of thermal selection in lizards. B. T. Firth and I. Belan
	Spontaneous torpor in captive dusky woodswallows (*Artamus cyanopterus*). Tracy A. Maddocks and Fritz Geiser
	Melatonin administration and reproduction in the marsupial *Antechinus stuartii*. Bronwyn McAllan* and Wendy Westman
	Effect of sodium depletion on the concentration of cAMP and the activity of Na^+/K^+-ATPase in the gills of crayfish *Cherax destructor*. Jiling Mo* and Peter Greenaway
	Handling stress and its effects on ventilation in two reptiles. S. L. Munns* and B. K. Evans

ASM

FRIDAY 3RD DECEMBER 1999

POSTERS	This total pigment went to market: Individual variation in myoglobin content in light muscle of farmed juvenile southern bluefin tuna *Thunnus maccoyii* (Castlenau). *Brian D. Paterson, Phillip Thomas, and Ross A. Smith
	Switching off the thermostat: thermoregulation by eastern skunk cabbage (*Symplocarpus foetidus*) Roger S. Seymour* and Amy J. Blaylock
	Why do lobsters recover oxygen uptake during prolonged air exposure? Harry Taylor* and Michelle Pritchard
	The physiological effects of ectoparasite infection of coral trout, *Plectropomus leopardus*. T. B. Turner*, M. B. Bennett and S. M. Bennett
	Pelvic limb anatomy of the red kangaroo and brush-tailed bettong. Koa Webster*, Matthew C. Raad and Terence J. Dawson
	The effect of glucoprivation on thermal physiology of *Sminthopsis macroura*: torpor or hypothermia? Wendy Westman*, Gerhard Körtner, and Fritz Geiser
	Locatisation of follistatin in testes of rat and brushtailed possum (*Trichosurus vulpecula*). Yin Xia* and J. R. McFarlane
	Effects of oxidative insult on red blood cells from high and low GSH sheep. Cheng-Gang Zou *, Nihal S. Agar and Graham Lloyd Jones
	Different mechanism for the glycerol induced haemolysis of human and sheep red blood cells. Cheng-gang Zou, Nihal S. Agar and Graham Lloyd Jones*

BBQ IN MARY WHITE COLLEGE GROUNDS 6.30 L

SATURDAY 4TH DECEMBER 1999

	Session One - Thyroid function Chair: Barbara Evans
9.00 - 9.20	Thyroid hormones & their effects: a new perspective. A. J. Hulbert
9.20 - 9.40	Baseline corticosterone, total and free thyroid hormones in juvenile salt water crocodiles (*Crocodylus porosus*). Caroline A. Shepherdley*, Samantha J. Richardson, Barbara K. Evans
9.40 - 10.00	Evolution of transthyretin structure and function. S. J. Richardson*, P. Prapunpoj, K. Yamauchi and G. Schreiber
10.00 - 10.20	Development of thyroid axis in lungfish. J. M. P. Joss.
10.20 - 10.40	Towards understanding thyroid hormone homeostasis in transthyretin null mice. Irna Grace T. Reytomas* and Samantha J. Richardson
10.40 - 11.10	*Morning tea*

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P Mac S O S O

SATURDAY 4TH DECEMBER 1999

	Session Two - Muscle Physiology Chair: Roger Seymour
11.10 - 11.30	Evolution and function of vertebrate sarcomeric myosins. Joseph F. Y. Hoh*, Michael Hsu and Han Qin
11.30 - 11.50	Characterization of myosin heavy chain isoforms of limb muscles of macropodids. W. H. Zhong*, C. A. Lucas and J. F. Y. Hoh
11.50 - 12.10	The effect of aestivation on the cardiovascular system of the striped-burrowing frog, *Cyclorana alboguttata.* Stephanie Myles* and Craig Franklin
12.10 - 12.30	Muscle-tendon interrelationships in the pelvic limb of turkeys, *Meleagris gallapavo*: Functional implications. R. B. Campbell* and M. B. Bennett
12.30 - 12.50	Effect of muscle receptor organ (MRO) ablation on tailflipping motor program in crayfish (*Cherax destructor*). Zen Faulkes *, Alisdair Daws & David MacMillan
12.50 - 14.00	*Lunch*
	Session Three - Endocrine Physiology Chair: Jean Joss
14.00 - 14.20	Developmental changes in activin C concentrations in the sheep. Peter Kearney*, and Jim McFarlane
14.20 - 14.40	Sexual dimorphism in ovine plasma concentrations of leptin. Kate Kauter, Tim O'Shea and Jim McFarlane*
14.40 - 15.00	Stress and tuna flesh. Philip M. Thomas*, John Carragher, Bruce Goodrick, Brian Paterson, Alistair Douglas and Alastair Smart
15.00 - 15.20	Physiological effects of capture and transportation in the coral trout, *Plectropomus leopardus.* T. B. Turner*, A. J. Bradley and M. B. Bennett
15.20 - 15.40	Adrenocortical responses to capture in northern tuatara (*Sphenodon punctatus punctatus*) from a rodent-free and a rodent-inhabited island. Claudine L. Tyrrell* and Alison Cree
15.40 - 16.10	*Afternoon tea*
	Session Four - Digestive Physiology Chair: Ian Hume
16.10 - 16.30	Adaptation of honeyeaters to a low-protein diet. L. R. (Rick) Allen* and Ian D. Hume
16.30 - 16.50	Do tammars take meals? The temporal distribution of feeding behaviour in captive and free ranging *Macropus eugenii.* R. G. Lentle*, K. J. Stafford, M. A. Potter and B. P. Springett
16.50 - 17.10	Digestion of eucalyptus pollen by two Australian nectarivorous psittacines. B. D. Gartrell*, S. M. Jones, L. B. Astheimer and R. N. Brereton
17.10 - 17.30	Selective stimulation and blockade of ß-adrenoceptors in kangaroo mandibular glands. A. Michel Beal
17.30 - 17.50	The effect of aestivation on the structure and function of the small intestine in the striped burrowing frog, *Cyclorana alboguttata.* Rebecca L. Cramp* and Craig E. Franklin

CONFERENCE DINNER "JITTERBUG MOOD" FROM 7.00 PM

Bring your Dinner ticket

SUNDAY 5TH DECEMBER 1999

	Session One - Reproduction Chair: Bill Buttemer
9.00 - 9.20	TO BrEed OR NOT TO BrEed some observations on breeding behaviour in the Tasmanian echidna, (*Tachyglossus aculeatus*). Niels A. Andersen* and Stewart C. Nicol
9.20 - 9.40	What is the timecourse of courtship in *Pteropus scapulatus*, little red flying foxes? G. M. O'Brien
9.40 - 10.00	Birth in the bandicoot, *Isoodon macrourus.* Robert T. Gemmell*, Colleen Veitch and John Nelson.
10.00 - 10.20	Birth in the brushtail possum, *Trichosurus vulpecula* (Marsupialia: Phalangeridae). Colleen E. Veitch* and Robert T. Gemmell
10.20 - 10.40	Expression of prostaglandin h synthase-2 in the endometrium and the yolk sac membrane of the tammar wallaby. L. T. Sebastian*, G. Shaw, G. E. Rice and L. J. Parry
10.40 - 11.10	*Morning tea*
	Session Two - Sodium Regulation Chair: Tony Hulbert
11.10 - 11.30	An overview of the natriuretic peptide receptor system in the gills of fishes. Tes Toop* and Brad Joblin
11.30 - 11.50	Characteristics of a natriuretic peptide guanylyl cyclase receptor in the hagfish. Will Callahan* and Tes Toop
11.50 - 12.10	The effect of water deprivation on atrial natriuretic peptide mRNA expression in the hopping mouse *Notomys alexis.* John Donald* and Rachel Heimeier
12.10 - 12.30	Immunolocalisation of NHE-like immunoreactivity in the gills of elasmobranchs. S. L. Edwards*, J. A. Donald and T. Toop.
12.30 - 12.50	Sodium and water usage by free-ranging robber crabs. Peter Greenaway
12.50 - 14.00	*Lunch*
	Session Three - Renal function Chair: Tes Toop
14.00 - 14.20	Kidney development in *Notomys alexis* and *Mus musculus domesticus* Rebekah King* and Juliet R. Roberts
14.20 - 14.40	Field water use in red (*Macropus rufus*) and eastern grey (*Macropus giganteus*) kangaroos. K. J. McTavish*, T. J. Dawson, A. Munn and J. Holloway
14.40 - 15.00	Aldosterone and avian species. Juliet R. Roberts
15.00 - 15.20	Control of kidney analogue function in land crabs - lessons in the evolution to life on land. Steve Morris*, Peter Greenaway, Agnieszka Adamczewska & Mark Ahern
15.20 -	*Afternoon tea and general meeting*

ABSTRACTS OF SPOKEN PRESENTATIONS

Adaptation of honeyeaters to a low-protein diet

L. R. (Rick) Allen[1]* and Ian D.Hume [2]

1 The Johnstone Centre, Charles Sturt University, PO Box 789, Albury NSW 2640
2 School of Biological Sciences, University of Sydney, NSW 2006

Part of the radiation of passerine birds was the adaptation of some ancestral insectivores to diets where carbohydrate partially or wholly replaced protein as an energy source. Specialist exploitation of carbohydrate-rich dietary niches can result in protein becoming a limiting factor. A reduced maintenance nitrogen requirement will be an adaptive advantage to species in such nitrogen-limited dietary niches, as is the case with arid zone macropods.

Data collected from three Honeyeater species (White-plumed Honeyeater, Brown-headed Honeyeater and Eastern Spinebill) yielded MNRs similar to a value calculated from the literature for non-passerines, and lower than values for all other passerines so far measured. Comparison with a wider range of species reported in the literature was restricted because inappropriate methods were used to estimate MNR in many cases, including the use of growing or reproducing birds and the assumption that maintenance of body mass necessarily coincides with maintenance of zero nitrogen balance.

MNR

TO BrEed OR NOT TO BrEed some observations on breeding behaviour in the Tasmanian echidna, (*Tachyglossus aculeatus*)

Niels A. Andersen and Stewart C. Nicol

Department of Anatomy and Physiology, University of Tasmania, G.P.O. Box 252-24, Hobart 7001, Tasmania, Australia.

Surprisingly few studies have dealt with reproductive physiology in the echidna since the first descriptions in the late eighteen hundreds. General consensus is that echidnas breed in late winter to early spring. Semon 1894 reported that in Queensland at the end of August most mature female echidnas were carrying an egg in the uterus or the pouch. In the last decade we have learned that echidnas in cooler habitats hibernate. This gives rise to several questions: how does hibernation and breeding interact and what is the thermal implication on reproductive success of breeding in mid winter? In recent years field studies have been undertaken mainly on Kangaroo Island, in Mt Kosciusko National Park and in Tasmania. Every time a new paper is published on the subject it seems to create new controversies. We will carry on the tradition and deal with many of these controversies and more from a Tasmanian perspective. We have been radiotracking breeding female echidnas over the last four breeding seasons. Some of these females were implanted with temperature loggers give us a continous temperature record during the time of breeding and rearing the young. This combined with the 1888, 1939 and 1978 published records on Tasmanian echidnas should finally solve the enigma.

The energetics of locomotion in vertebrates: After twenty years, where are the generalisations?

R.V. Baudinette

Department of Environmental Biology, University of Adelaide, Adelaide SA, 5005

Measurements of oxygen consumption have shown that it is metabolically cheaper for animals to swim than to run, despite having to move through a more dense medium. This attractive analysis was valid when the scaling relationships for swimming animals were dominated by data from fish but newer results from semi-aquatic mammals and birds have questioned its validity. Furthermore, recent data from mammals suggest that the costs for flying, swimming and running among specialists may be congruent. In this review the total cost of transport (COT) during sustainable swimming is analysed for amphibians and reptiles. The analysis questions whether a single line can represent the relation between COT for swimming and body mass. In order to examine the compatibility of economical running and swimming new data are presented for swimming toads and turtles, animals which have to move in both aquatic and terrestrial environments without apparent specialisation for either.

Selective stimulation and blockade of ß-adrenoceptors in kangaroo mandibular glands

A Michel Beal

School of Biological Sciences, University of New South Wales

Based on 3 species, eutherian salivary glands are believed to have $ß_1$-adrenoceptors only. Intracarotid (IC) infusion of noradrenaline, $ß_1$-selective adrenergic agonist, at 0.15 nmol/kg/min increased salivary protein, magnesium and bicarbonate, and decreased in osmolality, sodium, potassium and chloride. Concurrent IC infusions of noradrenaline and phentolamine (1.5 nmol/kg/min) had similar efficacy to noradrenaline alone whereas intravenous (IV) infusions of noradrenaline stimulated much smaller responses. Concurrent IC infusion of CGP20712A, $ß_1$-selective antagonist, was more effective than infusion of ICI118551, $ß_2$-selective antagonist, at blocking the changes in salivary composition caused by noradrenaline thereby demonstrating that the kangaroo mandibular, like eutherian glands, has $ß_1$-adrenoceptors. IC infusion of salbutamol, $ß_2$-selective agonist at 0.15, 0.3 and 1.5 nmol/kg/min caused qualitatively similar changes in salivary composition to IC-noradrenaline with 0.3 nmol being most similar quantitatively. Changes in salivary composition during IV-salbutamol were less than those for IC-salbutamol but the differences were small in comparison to those for IV- and IC-noradrenaline which could indicate that the response to salbutamol is mediated reflexly by a general increase in sympathetic tone triggered by the vasodilation associated with salbutamol administration. Concurrrent IV and IC infusions of CGP and ICI with IC-salbutamol showed that IC-ICI/IV-CGP was more potent than the reverse combination thereby demonstrating the presence of $ß_2$-receptors in the gland. Based on the changes in protein and osmolality, both endpieces and duct system have the two adrenoceptor subtypes.

The influence of age, organ mass, and testosterone on aerobic performance in house sparrows

W.A. Buttemer[1], C. Bech[1,2], M.A. Chappell[1,3], and L.B. Astheimer[1] .

1 Australian Flora and Fauna Research Centre, University of Wollongong
2 Dept. of Zoology, University of Science and Technology, Trondheim, Norway
3 Dept. of Biology, University of California, Riverside

We measured basal and peak aerobic metabolic rates and organ masses of juvenile and adult House Sparrows captured shortly after the breeding season. Neither BMR nor VO_2max was affected by gender, but juveniles had significantly higher BMR and lower VO_2max than adults. The pooled data revealed BMR to be positively correlated with four organ masses (gut, liver, kidney, and flight muscle) whereas VO_2max was positively correlated with mass of heart and flight muscles. The higher BMR in juveniles is consistent with their significantly larger central organ masses compared to adults, but their smaller VO_2max is probably related to both qualitative and quantitative differences in heart and flight muscles. We also conducted a pilot study to examine the effects of testosterone on basal and peak aerobic metabolism. All birds showed a decline in body mass, basal, and peak metabolic rates during their 9 weeks in captivity. Birds given exogenous testosterone (T) tended to show less of a reduction in VO_2max and aerobic scope during this period. T-treated birds had significantly larger flight and leg muscle masses than control birds but heart muscle masses were indistinguishable between the two groups.

Characteristics of a natriuretic peptide guanylyl cyclase receptor in the hagfish

Will Callahan* and Tes Toop

School of Biological and Chemical Sciences, Deakin University, Geelong VIC 3217

Mammalian natriuretic peptide hormones counter hypertension by stimulating natriuresis, diuresis and vasodilation. Natriuretic peptide receptors are of two types. Biologically 'silent' receptors regulate plasma concentrations of natriuretic peptides by removing them from the circulation. In contrast, guanylyl cyclase (GC) natriuretic peptide receptors mediate the effects of natriuretic peptides by catalysing the production of cGMP. The GC activity of mammalian natriuretic peptide receptors is regulated by the binding of the ligand to the extracellular domain and also by the binding of ATP to an intracellular allosteric site. GC activity also differs depending on which cofactor (Mn^{2+} or Mg^{2+}) is present. This study demonstrates the existence of a GC natriuretic peptide receptor in the hagfish, indicating that GC natriuretic peptide receptors are an ancestral vertebrate characteristic. Similar to mammals, the GC activity of hagfish natriuretic peptide receptors appears to be regulated by ATP, Mg^{2+} and Mn^{2-}. ATP stimulates cGMP accumulation in the presence of Mg^{2+} but is inhibitory in the presence of Mn^{2+}. Although ligand-dependent GC activity is proportionally greater in the presence of ATP and Mg2+ than in the presence of ATP and Mn^{2-}, basal GC activity is lower in the presence of Mg^{2-} than in the presence of Mn^{2+}.

Muscle-tendon interrelationships in the pelvic limb of turkeys, *Meleagris gallapavo*: Functional implications

R. B. Campbell* and M. B. Bennett

Department of Anatomical Sciences, University of Queensland, St. Lucia 4072.

Muscles and tendons have long been known to play an important role in the storage and release of mechanical energy during running in animals. The turkey presents a unique model where the muscle-tendon interrelations involve calcified tendons, thereby altering the 'normal' muscle tendon relationship.

Analysis of the morphometric measures of 13 crural muscles was investigated. Tibiotarsus and tarsometatarsal dimensions and moment arms did not change after about 20 weeks of age. Muscle fibre lengths and their pinnation angles did not alter with age, although body mass doubled by 80 weeks. This doubling of body mass was paralleled by increases in crural muscle mass, resulting in a doubling of muscle force capacity. Fibre lengths were predicted to change with increasing age as the stiffness of their attached tendons increased with mineralisation, and tendon modulus approximately trebled over this time. The lack of change in fibre length was unexpected. Increasing tendon stiffness and constant muscle fibre length implies a reduction in RoM at distal joints. However, as tendon modulus increases, the amount that muscle fibres would have to shorten to generate movement of a joint, against a standard resistance, would be reduced. This may be of energetic benefit for those muscles that resist ankle extension during locomotion. However, tendon mineralisation has negative connotations for elastic strain energy storage in general.

The effect of aestivation on the structure and function of the small intestine in the striped burrowing frog, *Cylorana alboguttata*

Rebecca L. Cramp* and Craig E. Franklin

Department of Zoology and Entomolgy, University of Queensland, Brisbane, Australia

The effect of prolonged starvation during aestivation on the structure and function of the small intestine was examined in the striped burrowing frog, *Cylorana alboguttata*. Following 12 weeks of aestivation, there were significant changes to the structure of the small inestine, including a reduction in longitudinal fold height, a reduction in enterocyte cross-sectional area, and a reduction in microvilli height. The functional capacity of the small intestine was, however, not affected by 12 weeks of aestivation. This was evidenced by a maintenance of the mass-specific metabolic rate of the isolated small intestine and the maintenance of the nutrient transport ability of the small intestine. This study clearly indicates that while structural elements of the small intestine may be significantly reduced during aestivation to accrue energy savings, the functional capacity of the gut is potentially not compromised.

The role of fur: polar bears versus koala 'bears'

T. J. Dawson*, S. K. Maloney and Koa Webster

School of Biological Science, University of New South Wales, Sydney 2052, NSW, Australia

In polar bear fur it has been suggested that individual hairs act as light guides to transmit solar radiation to the skin, thereby trapping heat. This was recently called a polar myth. We have been examining of koala fur; koalas, also put up with extremes of weather. We got samples of polar bear fur and felt that a comparison with the koala would provide insight into the properties of fur. The reflectance of the two furs are different. For the whole solar spectrum, reflectance is 9.5% for the koala and 66% for the polar bear. Both have low conductances, ie are good insulators, and are very little affected by wind. In both species, at 1m/s of wind about 19% of incident solar radiation ends up at the skin as heat; this decreases at 10 m/s to 7-10%. So, there is very little difference between the coats of these 'bears' in the protection afforded against heat loss and solar loads. The notion that the hairs act as light guides to give the penetration of much solar heat to the skin is not true.

The koala and the polar bear both have furs that are effective in broad thermal terms. Also, they are effective in terms of crypsis. Both mammals are very hard to see in their peculiar environments.

Polar bear fur purchase only about $\frac{1}{2}$ fur thickness – more likely to reach skin

The effect of water deprivation on atrial natriuretic peptide mRNA expression in the hopping mouse *Notomys alexis*

John Donald* and Rachel Heimeier

School of Biological and Chemical Sciences, Deakin University, Geelong

The spinifex hopping mouse, *N. alexis*, is capable of tolerating an absence of free water for prolonged periods by excreting a concentrated urine and utilising metabolic water. We are interested in the role of the natriuretic peptide system in the neuroendocrine control of fluid balance in *N. alexis*, because the system increases water and salt excretion in mammals; such regulation in *N. alexis* would not support survival in xeric environments. In this study, hopping mice were subject to water deprivation for either seven or twenty-eight days, in parallel with control mice that had access to water. All animals were fed a birdseed diet. In all animals, haematocrit, plasma osmolarity, and tissue water content were determined. The expression of atrial natriuretic peptide (ANP) mRNA was determined in the heart and kidney, using quantitative analysis of RNA dot blots, and homologous ANP and B-actin cDNA probes. Interestingly, cardiac ANP mRNA expression increased in both 7-day and 28-day experimental animals compared to controls, but the renal mRNA expression decreased. These data suggest that the cardiac ANP biosynthetic pathway is not linked to fluid volume regulation, but that the renal expression of ANP was down-regulated, probably to reduce the excretion of water and salt.

Immunolocalisation of NHE-like immunoreactivity in the gills of elasmobranchs

S. L. Edwards*, J. A. Donald and T. Toop

School of Biological & Chemical Sciences, Deakin University, Geelong, Victoria

Na^+/H^+ exchange has been implicated in models of ion transport across the branchial epithelium of fishes. In this study, we have used polyclonal antibodies raised against NHE2 and NHE3 to demonstrate NHE-like immunoreactivity in the gills of 4 elasmobranch species. NHE2-like immunoreactivity was present in 2 discrete populations of cells within the gills of the Banjo ray (*Trygonorrhina fasciata*) and Eagle ray (*Myliobatis australis*). The larger populations consisted of large ovoid cells located in the junction between the filament and secondary lamellae and were Na^+/K^+-ATPase immunoreactive. The second population of cells, displaying NHE2-like immunoreactivity were smaller in size and displayed no evidence of Na^+/K^+-ATPase colocalisation. In the Gummy shark (*Mustelus antarcticus*) NHE3-like immunoreactivity was demonstrated in a single population of large ovoid cells located in the junction between the filament and secondary lamellae which were also Na^+/K^+-ATPase immunoreactive. NHE2 and NHE3-immunoreactive cells were also present in 2 separate populations of cells within the branchial epithelium of the angel shark (*Squatina australis*). Neither population of cells demonstrated Na^+/K^+-ATPase colocalisation and appeared to be sub-epithelial in origin. This study provides valuable anatomical evidence to support the existence of a functional Na^+/H^+ exchanger in the branchial epithelium of elasmobranchs.

Effect of muscle receptor organ (MRO) ablation on tailflipping motor program in crayfish (*Cherax destructor*)

Zen Faulkes *, Alisdair Daws ** & David Macmillan

Department of Zoology, University of Melbourne, Royal Parade, Parkville, VIC, 3052, Australia ** Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, 43403, U.S.A.

Muscle receptor organs (MROs) are abdominal proprioceptors found in many decapod crustaceans, including Australian crayfish ("yabbies;" *Cherax destructor*). MRO sensory neurons fire in response to abdominal flexion. Traditionally, MROs have been thought to be involved primarily in mediating local resistance reflexes, but work in our lab indicates that this may not be their main function (at least in yabbies). MRO sensory neurons have axons that run the entire length of a crayfish's nervous system, which suggests MROs may help coordinate whole body movements. One such "whole body" behaviour is tailflipping, an escape response in which the abdomen is flexed and extended repeatedly. Surgical ablation of the MROs significantly alters tailflipping behaviour. The uropods (appendages of the last tail segment) close less in individuals in which the MROs were removed than those in which the MROs were intact. We are currently videotaping crayfish tailflipping while recording electromyograms (EMGs) from uropod adductor muscles simultaneously. This should help us to pinpoint which uropod motor neurons are receiving MRO input and how MRO ablation affects the tailflipping motor program.

Cloacal ventilation and dive performance of the Fitzroy River turtle, *Rheodytes leukops*

Craig E. Franklin and Toni Priest*

Department of Zoology and Entomology The University of Queensland, Q 4072

Rheodytes leukops is a bimodally breathing turtle obtaining oxygen via lungs and modified cloacal bursae. Histological examination has revealed bursae that are lined with multi-branching papillae that are well vascularised with microvilli present on the epithelium.
Electromagnetic flow probes were used to establish the rate and volume of ventilation of the cloacal bursae. The effect of PO_2 on cloacal ventilation was also investigated.
Depth and temperature sensitive data loggers were used in field studies to explore the dive performance of *R. leukops* under natural conditions. *R. leukops* displays a bimodal activity pattern with peaks centered around dawn and dusk. The maximum dives recorded during two separate field deployments were 63.8 and 91.7 hrs. Seasonal patterns and response to flooding events will be discussed.

Digestion of Eucalyptus Pollen by two Australian nectarivorous psittacines

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The importance of pollen as a source of protein to nectarivorous psittacines has been controversial. Evidence supporting its importance has come from field studies, however, an experimental study of *Eucalyptus* pollen digestion in *Trichoglossus haematodus* showed less than 7% of ingested pollen is digested in adults birds. That study concluded that pollens do not furnish a significant source of protein. The aim of our study was to investigate the digestion of pollen by two nectarivorous parrots, the swift parrot *Lathamus discolor* and the musk lorikeet *Glossopsitta concinna*. We provided both fresh and frozen flowers for birds as sole food for a period of four hours in individual cages. All birds voluntarily consumed both nectar and pollen from flowers with a different feeding action noted for each. The extent of pollen digestion was measured by analysis of stained faecal smears for pollen morphology taken at two, three and four hours. This was compared to a stained sample of pollen collected from flowers immediately prior to each trial. Initial results show a proportion of the pollen was digested by both species, indicating that pollen plays a major nutritional role for these parrots as a source of high quality and easily harvested protein.

Torpor in free-ranging owlet-nightjars, *Aegotheles cristatus*

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In Australia knowledge about torpor in free-ranging birds is restricted to anecdotal reports. As some northern hemisphere caprimulgiform birds (nightjar relatives) are known to use torpor for energy conservation, we determined whether free-ranging Australian owlet-nightjars (50 g) enter torpor on the cool-temperate New England Tablelands. We tracked 11 of these nocturnal birds carrying temperature-sensitive transmitters mounted in backpack style for measurement of skin temperature (T_{skin}); an internal transmitter was implanted into one additional bird to measure core body temperature (T_b). One captive bird was fitted with both internal and backpack transmitters to determine T_b - T_{skin} differentials. All birds measured in winter (n=6) entered torpor. Torpor was frequently used and bouts usually lasted from dawn to midday, but some birds re-entered torpor in the afternoon. Night torpor was rarely observed. The lowest T_{skin} recorded was 19.6 °C and the lowest T_b was 22.4 °C. Our results suggest that heterothermy plays a pivotal role in the survival strategy of owlet-nightjars and may explain why they can afford to be sedentary despite a fluctuating supply of insects. The frequent use of torpor suggests that heterothermy in small Australian birds is more common than is currently believed.

Birth in the bandicoot, *Isoodon macrourus*

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In this study we filmed birth in the bandicoot and tried to ascertain the senses that allow the young to transfer from the uterus to the pouch. The newborn marsupial has rudimentary vestibular, olfactory, and mechanoreceptors, and these structures may aid the newborn in the journey to the pouch. Before birth the bandicoot lay on one side with one hind leg facing upwards. Young were born singularly or in groups of up to four young, the mother licking the young vigorously to remove the membranes. Unlike the possum the pathway of the newborn of the bandicoot was mainly downwards with the newborn only having to travel about 1 cm to reach the pouch. The newborn of the bandicoot do not have a definite crawl to the pouch, they have a snake-like wriggle down a moist 1 cm pathway between the urogenital sinus and the pouch. The mother bandicoot lies on one side then on the other positioning the pouch so that the young nearly "fall" into the pouch. It is obvious that there are several marsupial "birth positions". However it is difficult to determine if olfaction and or gravity assist the bandicoot young to reach the teat.

umbilical cord cut by mother about 1 h after birth

Sodium and water usage by free-ranging robber crabs

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Water and sodium exchange of the terrestrial anomuran *Birgus latro* were examined in field situations on Christmas Island. Turnover of water (48 $mL \cdot kg^{-1} \cdot d^{-1}$) and sodium (7.8 $mmol \cdot kg^{-1} \cdot d^{-1}$) were unexpectedly high and considerably above values for animals in laboratory situations (X 2-3 for water and X 48 for Na). Values for clearance of the filtration rate marker ^{51}Cr-EDTA provided confirmation of high turnovers of both water and sodium. The bulk of the water and sodium effluxes from the animals could be accounted for in the excretory fluid. Intake of water is primarily by drinking and as only fresh water is available most of the intake of sodium must come from the food. The crab is believed to feed primarily on high energy fruits and seeds and to a lesser extent to prey on other crabs and scavenge carcases. As the sodium content of most seeds is very low it seems likely that animal material is a frequent and substantial component of the diet.

Evolution and function of vertebrate sarcomeric myosins

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In mammals, 10 isoforms of sarcomeric myosin heavy chains (MyHCs) are known: developmental isoforms (embryonic, foetal), fast skeletal isoforms (2A, 2X, 2B) extraocular-fast, cardiac isoforms (α, β/limb slow) superfast and extraocular slow-tonic. Such heterogeneity generates muscle fibres with a wide range of speed, power and economy. Genes for the first 6 listed are tightly linked in human chromosome 17, while genes for cardiac isoforms are tightly linked in chromosome 14. The complete nucleotide sequence for these 8 human genes are available. We have cloned and sequenced the full length of the cat superfast MyHC. This gene in humans is localised at chromosome 7. A phylogenetic tree of representative known vertebrate MyHC amino acid sequences revealed that superfast MyHC was the first to diverged from an invertebrate ancestral MyHC. Furthermore, mammalian cardiac MyHCs are more closely related to avian slow skeletal MyHC than to avian cardiac MyHC, suggesting that during evolution the mammalian heart adopted the use of the limb slow β MyHC) and its duplicate (α MyHC). The extraocular-fast MyHC shares a common ancestor with the fast and developmental group, being phylogenetically the oldest within this cluster. It has a much more recent evolutionary origin than superfast MyHC.

Effects of a helium/oxygen atmosphere on thermoenergetics in the sugar glider, *Petaurus breviceps*

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For nearly 50 years researchers have used helox (79 % helium:21 % oxygen) for thermobiological studies, primarily because helium is thought to be metabolically inert and to produce no adverse effects other than increasing thermal conductance. However, these assumptions have been questioned. As basal metabolic rate (BMR) represents maintenance energy requirements for vital body functions, any physiological effects of helox should be reflected in changes of BMR. In the present study, sugar gliders were subjected to both air and helox atmospheres over a wide range of T_a, including the thermoneutral zone (TNZ), to determine: 1) whether helox has any influence other than on thermal conductance, and 2) the maximum metabolic rate and thermal limits of this species. Although thermal conductance in the TNZ increased in helox, BMR was similar in air and helox (0.57 ± 0.06 and 0.55 ± 0.03 ml O_2 g^{-1} h^{-1}, respectively). The TNZ in helox, however, was shifted upward by about 3 °C. Below the TNZ, sugar gliders were able to withstand an effective T_a of -24.7 ± 7.3 °C, with a maximum metabolic rate of 3.14 ± 0.36 ml O_2 g^{-1} h^{-1}. Similarities of BMR in air and helox suggest that helox only increases thermal conductance and consequently represents a useful tool for thermal physiologists.

Thyroid hormones & their effects: a new perspective

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The thyroid hormones are very hydrophobic iodothyronines and those that exhibit biological activity are T4, T3, rT3 and 3,5-T2. At physiological pH, these hormones are amphipathic and thus associate with membranes. In adult vertebrates, unbound T4 and T3 are in the picomolar range whilst protein-bound T4 and T3 are maintained in the nanomolar range. The function of thyroid hormone-binding plasma proteins appears to be ensure a relatively even distribution throughout the body. The various iodothyronines are produced by membrane bound cellular deiodinase enzyme systems, of which three types are found in vertebrates. It is proposed that there are a number of modes of action of these hormones. 1) The nuclear receptor mode is especially important in the thyroid hormone axis, 2) Thyroid hormones associate with membranes and rigidify these membranes, 3) consequently altering the acyl composition of membrane bilayers. Thyroid hormone effects on metabolism are due primarily to membrane acyl changes. During development, vertebrates show a surge in T4, and a distinctive profile in the appearance of the deiodinase enzymes and nuclear receptors. Evidence from analogues, as well as from "knockout" mice, and data from the early 1960s is re-examined in light of these proposed mechanisms.

Development of the pulmonary surfactant system in the snapping turtle, *Chelydra serpentina*

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Pulmonary surfactant (PS), a mixture of phospholipids (PL), neutral lipids and proteins, lowers surface tension within the lung, reducing lung compliance and aiding the removal of lung fluid at birth. In mammals, the PS system develops towards the end of gestation, marked by an increase in PL saturation and the appearance of surfactant proteins in lung and amniotic fluid. Here, we examined the composition of PS lipids and the expression of the surfactant protein, SP-B and Thyroid Transcription Factor-1 (TTF-1) in the developing lung of the snapping turtle, *Chelydra serpentina*. Lung tissue and lavage were collected from embryos at day 56, day 61 (pre-pipped), day 61-66 (post-pipped) and 61-69 (post-hatched) of incubation. Total PL and disaturated phospholipid (DSP) increased throughout incubation, resulting in an increase in the DSP/PL ratio. TTF-1, a regulator of gene expression of surfactant proteins and cell differentiation in mammals, was detected in epithelial cells of the lung throughout development using immunohistochemistry. Similarly, SP-B was detected in aggregates of cells within the respiratory epithelium at all stages sampled. This demonstrates that the regulating factor and the surfactant proteins are highly conserved within the vertebrates. Like mammals, the turtle surfactant lipids develop and mature towards the end of incubation, however the relative timing of development is greatly truncated, probably due to the metabolic demands of the embryo and precocial nature of the young.

Development of thyroid axis in lungfish

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The thyroid of the lungfish, *Neoceratodus forsteri*, is very rudimentary at hatching. It comprises 2-3 small follicles of inactive appearance. By 10 months of age, thyrotropes can be recognised in the anterior pituitary. Treating lungfish at this age with methimazole (inhibits iodine incorporation into thyroid hormones) does not enhance I- uptake by thyroid follicles. Lungfish have been tested for pituitary/thyroid interaction in this way up to 12 months of age at which stage they do not show any interaction, although the numbers of identifiable thyrotropes and the numbers of thyroid follicles are still increasing. Experiments involving exposure of lungfish 8-11 months old to excess thyroid hormones or methimazole produce considerable differences in appearance of thyroid but not the pituitary. It must be concluded from these results that a mature pituitary/thyroid axis is not present in lungfish up to 12 months of age. At the target tissue level, lungfish at this age express primarily the more juvenile alpha-receptors. They also show greater activity of the 5D than the 5'D deiodinase enzyme, indicating that deiodinating activity is primarily degrading thyroid hormone rather than converting thyroxine to the more active triiodothyronine. The significance of these data will be discussed.

Sexual dimorphism in ovine plasma concentrations of leptin

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Leptin, a recently discovered hormone secreted mainly from adipose tissue, was first described as a regulator of adiposity, food intake and energy metabolism. It is now apparent that leptin physiology is much more complex and is likely to play an important role in many other systems including reproduction, haematopoiesis and immunity. Leptin levels have been shown to be well correlated with body fat in both humans and rodents. Leptin is therefore likely to be an important humoral signal to the central nervous system on body composition and regulation of food consumption. A number of studies have reported that leptin levels in rodents and humans are generally lower in males than females. Using our recently developed assay for ovine leptin we investigated this phenomena in merino sheep. Leptin levels in ram plasma samples (3.1 ± 1.3 ng/ml) was significantly lower than the levels measured in ewes (6.0 ± 2.9). This data supported the view of previous workers that testosterone suppressed leptin secretion in males, however leptin levels in male castrate sheep were not significantly different (3.3 ± 0.4) from the levels in rams. Further studies have shown that castration of rams does not alter plasma leptin levels in the short or long term. This data suggests that rather than testosterone suppressing leptin levels in males, oestrogens may stimulate its production in females.

Developmental changes in activin C concentrations in the sheep

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Activins are dimeric molecules which belong to the transforming growth factor beta (TGF-ß) family of growth factors. These molecules were originally identified as FSH stimulating factors in pituitary cell cultures, and have since been implicated in a wide range of other physiological processes including erythropoesis, reproduction, cell differentiation and growth. The presently identified bioactive molecules of activin, have been characterised as either homo- or heterodimers consisting of inhibin $ß_A$ and $ß_B$ subunits. mRNA for a proposed third subunit, $ß_C$, has been isolated from murine and human liver. The aim of this study was to develop an immunoassay capable of detecting the putative activin C protein and elucidate a potential role in liver development. An antiserum (#310) was raised in sheep against a $ß_C$ peptide. This peptide was also used as both tracer and standard in conjunction with the antisera, to develop a $ß_C$ specific RIA. The assay was used to measure activin C like protein in homogenised ovine liver taken from maternal and foetal animals at different stages of gestation. Preliminary results indicate little variation of activin C in ovine maternal liver across gestation (29 ± 2.5 nM/g), and undetectable levels in foetal liver.

Kidney development in *Notomys alexis* and *Mus musculus domesticus*

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Gestation period and time to weaning are longer in *Notomys alexis* than in *Mus musculus domesticus*. It was hypothesised that these longer development times in *Notomys* represent adaptations to arid environments. Kidney structure was studied in both *Notomys* and *Mus* from birth to 80 days of age. *Mus* grew larger than *Notomys* and had larger kidneys and a higher percentage kidney weight. The total kidney, cortical and medullary areas in mid-sagittal section were greater for *Mus* than *Notomys*. For the papillary area, *Mus* was larger up to 70 days of age, but by 80 days of age, papillary area was the same for the two species. Relative medullary area and percent medullary area were greater in *Notomys* by 70 days of age. Percent papillary area was quite different between the two species, being significantly greater for *Notomys* from 40 days of age. Numbers of glomeruli per kidney and the relative proportions of cortical and juxtamedullary glomeruli were similar for both species although glomerular diameter was greater in *Notomys* by 70 days of age. It appears that *Notomys* are born with kidneys at a later stage of development and that initial postnatal development is slower than in *Mus*. However, the extent of development of the papilla is greater in *Notomys* by the time the animals reach sexual maturity.

Do tammars take meals? The temporal distribution of feeding behaviour in captive and free ranging *Macropus eugenii*

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The temopral distribution of feeding behaviour in captive and free ranging *Macropus eugenii* is discussed from a viewpoint of testing the validity of the assumptions of reactor theory, in which feeding behaviour is assumed to stabilise foregut content and digestive conditions, versus those of optimal foraging theory, in which digestive physiology is assumed to compensate for temporal irregularities of food intake.

Gas exchange in marsupial neonates: the role of the skin

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Marsupials are a unique group, distinguished from their eutherian counterparts by a short gestational length and prolonged period of lactation. As a consequence, the minute young is born at an immature stage of 'embryonic' development. The lungs are only at the terminal air-sac stage of development at birth and until recently were considered the primary site for gas exchange. It is now known that in the Julia Creek dunnart (*Sminthopsis douglasi*) the neonate is not breathing at birth and the skin supplies upwards of 95% of the gas exchange requirements. In contrast, the neonatal tammar wallaby (*Macropus eugenii*) is breathing at birth, though the spirometric record indicates that the lung is maintained inflated at the end of inspiration. The skin of the newborn tammar wallaby, however, still contributes some 30% of the total oxygen exchange. The contribution of the skin in both species decreases gradually following birth. The marsupial neonate's ability to obtain O_2 through the skin as an alternative site to the lung could be critical for survival, if not essential, during the first few days of pouch life. The impact of a very small body size and the immaturity of the marsupial neonate on ventilation are discussed. Supported by grants from the ARC.

Field water use in red (*Macropus rufus*) and eastern grey (*Macropus giganteus*) kangaroos

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It is frequently suggested that the provision of drinking water has facilitated the movement of eastern greys into arid areas and the habitat of red kangaroos in recent times. In a detailed comparative study we examined the functional water relations, feeding patterns and behaviours of red and eastern grey kangaroos in a large enclosure during late summer at Fowler's Gap in far western NSW. The aim was to see if the reported changes in distribution were supported by differences in physiology. There was no significant difference in the daily water turnover of the two species but red kangaroos produced a more concentrated urine, which was supported by kidney anatomy. Observations of microhabitat choice during the day replicated natural conditions where eastern greys seek dense shade of large trees, while red kangaroos rest in the sparser shade of small shrubs and long grass. A greater proportion of red kangaroos stayed in the sun throughout the day. This suggests that the red kangaroos were using the water saved by producing a more concentrated urine for thermoregulatory mechanisms. Also, the red kangaroos may be obtaining a greater amount of water from their diet. Eastern greys visited the water trough an average of once every two days while only half the red kangaroos visited during a four day period. These results support the idea that the recent movement of eastern greys into the area is associated with the provision of drinking water.

Control of kidney analogue function in land crabs - lessons in the evolution to life on land.

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The invasion of land by decapod crustaceans has occurred in several separate lineages, of both marine and freshwater origin. In addition to respiratory gas exchange, acid-base, water and nitrogen balance terrestrial animals must maintain salt homeostasis. This requires either the amelioration of, or compensation for, the loss of salts in the urine. The decapod crustacean antennal gland produces primary urine as a filtrate of the blood, analogous to the vertebrate kidney glomerulus. Aquatic crabs can usually recover lost salts by active transport of ions from the water but for terrestrial crabs this represents an unacceptable loss. Both brachyuran and anomuran land crabs are able to reprocess their urine by passing some or all of the primary urine over the gills where required salts can be reabsorbed. Thus land crabs utilise a filtration-resorbtion kidney analogue The terrestrial anomuran Robber crab (*Birgus latro*) and the brachyuran Christmas Island red crab (*Gecarcoidea natalis*) are commonly found some distance from the ocean and must utilise freshwater for drinking. The food; leaves and fruit for red crabs, and fruit, nuts and other crabs for robber crabs, represents the only significant source of salt. The water and salt intake of land crabs is thus variable and inconstant. It seems necessary that these animals possess sophisticated control mechanisms to precisely mediate the extent and duration of salt reclamation from the urine. Both *B. latro* and *G. natalis* utilise blood-borne monoamines as primary messengers to mediate branchial Na/K-ATPase activity and NaCl uptake. In *B. latro* dopamine functions as a primary messenger, mediated by cAMP as a cellular second messenger, to down-regulate pumping. In *G. natalis* serotonin (5-HT) acts to up-regulate the same system but does not require cAMP as a second messenger. While the two species appear to utlise very similar physiological processes for managing salt balance, the cellular physiology is different. This reflects the separate and distinct evolutionary lineages of these land crabs.

The ventilatory response to CO_2 in reptiles

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Despite decades of research on reptilian ventilation, the response to CO_2 in these animals remains puzzling. Breathing high concentrations of CO_2 can trigger a range of responses in reptiles including an increase in minute ventilation, no change, or even a decrease in minute ventilation. To complicate matters further, the wide variety of species, body temperatures, experimental protocols and techniques used makes drawing general conclusions from literature difficult. In this study the ventilatory response to hypercapnia was measured in three species of reptiles using the same methods and protocol. The species (*Crocodylus porosus*, *Physignathus lesueurii* and *Chelodina longicollis*) were chosen to represent three of the major groups of extant reptilians. The three groups of reptiles displayed dramatic differences in their ventilatory response to hypercapnia, however some general conclusions can be drawn. At least part of the difference in responses to CO_2 appears to be due to the presence (or absence) of upper airway chemoreceptors. These upper airway chemoreceptors, capable of detecting high concentrations of CO_2 in inhaled air and of triggering a decrease in ventilation, are present in lizards and snakes, but not in crocodilians.

The effect of aestivation on the cardiovascular system of the striped-burrowing frog, *Cyclorana alboguttata.*

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This study investigated the means by which the cardiovascular system undergoes compensations to conserve energy during the aestivation process in the striped burrowing frog, *Cyclorana alboguttata,* a cocoon forming species. No differences could be observed in the haematocrit or in the structure of either the heart or the vasculature at the gross anatomical level. However, the relative heart mass was significantly reduced by 28.2 %. The ultrastructure of the heart was significantly changed in the aestivators by a decrease in mitochondrial density (38.4 %) and an increase in the glycogen and lipid densities (38.4 and 50 % respectively). Heart rate, f_H, was significantly reduced in aestivating animals from resting levels. Disturbance increased the heart rate of aestivating animals but the rate dropped extensively over a 24 h period. In vitro preparations showed that contractile force was also significantly lower in aestivating animals. These results indicate that the cardiovascular system is making compensations during aestivation in order to conserve energy by accumulating energy liberating compounds in the myocardium and by reducing the cardiac output of the heart.

What is the timecourse of courtship in *Pteropus scapulatus*, little red flying foxes?

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In *Pteropus poliocephalus*, the greyheaded flying fox, the four months before mating are characterised by well described courtship behaviours. Broadcasting (scent dispersion) begins when territories and mates are being selected from Dec onwards, grooming and screaming begin in Jan, and increase in Feb then continue through the peak mating period, April-May. The present study aimed to define the timecourse, of courtship by *P. scapulatus*, the little red flying fox, who mate Nov-Dec. The hypothesis of the study was: that there is no difference between *P. scapulatus* and *P. poliocephalus* in the temporal relationship of courtship to mating. Data were collected from a breeding group (minimum 4 adult males, 10 adult females) housed in an outdoor pen in Brisbane. Durations of behaviours were: (i) aural stimulation (screaming) - Oct-Dec; (ii) olfactory stimulation (broadcasting) - Nov-Dec; (iii) oro-genital grooming of female by male - Nov-Dec. For *P. scapulatus* most courtship behaviours increased substantially in frequency and duration only at the onset of the mating season and continued for 2 months, Nov-Dec. In contrast *P. poliocephalus* courtship begins 4 months before mating with high frequencies extending over 4 months, Feb to May. This constitutes a major difference between species in the temporal relationship of courtship to mating.

Thermal and neural control of surfactant secretion from type II cells of the fat-tailed dunnart, *Sminthopsis crassicaudata*

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Pulmonary surfactant, a mixture of lipids and proteins, is synthesised in alveolar type II cells and secreted into the lining of the lung in response to ventilation, temperature changes and autonomic neurotransmitters. This study examines the thermal and neural control of surfactant secretion by type II cells isolated from the heterothermic marsupial, Smin*thopsis crassicaudata*. Both adrenergic and cholinergic agonists stimulated secretion at warm (37 °C) and cold (18 °C) incubation temperatures. Isoproterenol significantly increased phosphatidylcholine secretion after 2h from 9.5 % to 10.5 % at 37 °C ($p=0.02$) and from 6.9 % to 7.5 % at 18 °C ($p=0.03$). The cholinergic agonist, carbamylcholine chloride also increased secretion from 9.6 % to 11.9 % at 37 °C ($p=0.02$) and from 6.9 % to 7.4 % at 18 °C ($p=0.01$). Temperature affected the rate of secretion from type II cells (e.g. basal secretion: 9.6 % at 37 °C vs. 6.9 % at 18 °C, $p=0.02$), but the change in secretory rate between 37 and 18°C was less than expected if due to temperature alone ($Q_{10}=1.3$). The surfactant secretory pathway is therefore modulated by factors other than temperature. The response of type II cells to agonists remained the same at both temperatures. For example, after 2h, adrenergic-stimulated secretion was 116.2 % and 115.7 % of basal secretion at 37 and 18 °C, respectively. Therefore, in dunnarts there does not appear to be a switch between adrenergic and cholinergic stimulation during torpor at the cellular level.

Towards understanding thyroid hormone homeostasis in transthyretin null mice

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Transthyretin is one of three plasma proteins that binds thyroid hormones and has been proposed to have a role in establishing appropriate distribution of thyroid hormones between aqueous and lipid compartments. In mammals transthyretin is synthesised in the liver, choroid plexus and retina. Transthyretin null mice were purchased and a colony was established. The aim was to confirm by PCR of genomic DNA, sequencing of PCR products, Northern Blot analysis and Western Blot analysis that transthyretin gene was interrupted. Another aim was to identify the minimum challenge to thyroid hormone homeostasis necessary to cause hypothyroidism in wildtype and transthyretin null mice. Raised levels of thyroid stimulating hormone (TSH) is indicative of hypothyroidism. The transthyretin null mice were found to have an interrupted transthyretin gene with an insert more than 200 base pairs larger than described by Episkopou et. al (1993). Transthyretin mRNA was not detected by Northern analysis of RNA preparations from liver or brain. Transthyretin was not detected in serum. Propylthiouracil (PTU) inhibits thyroid hormone synthesis. 0.1 % PTU was administered in drinking water and levels of TSH were measured by a TSH immunoradiometricassay. Transthyretin null mice had a significant increase serum TSH level compared to wildtype mice, after 3 weeks.

Evolution of transthyretin structure and function

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Transthyretin is a protein involved in extracellular distribution of thyroid hormones (THs) in vertebrates. In mammals, birds and reptiles it is synthesised by the choroid plexus (blood-cerebrospinal fluid barrier), secreted into the cerebrospinal fluid, and is involved in the transport of TH from the blood into the brain. Within the cerebrospinal fluid, most TH is bound to transthyretin. In adult birds, eutherians and diprotodont marsupials, transthyretin is synthesised by the liver, and secreted into the blood, creating a circulating pool of thyroid hormone. We detected transthyretin synthesis in liver of metamorphosing amphibians. Others reported transthyretin in serum from smolting sea bream. Transthyretins from all vertebrates have high amino acid sequence identity, suggesting that a "pre-vertebrate transthyretin precursor" molecule could have existed. Database searches revealed 5 open reading frames (ORFs) from *C. elegans, E. coli, S.pombe* and *S. dublin* which have high amino acid sequence similarity to transthyretins. According to the rules of Doolittle, these are probably transthyretin homologues. Kyte-Doolittle hydophobicity plots of these ORFs revealed similar profiles to transthyretins. Recombinant transthyretins from reptiles and amphibians were synthesised in the yeast *Pichia pastoris*. These transthyretins had higher affinity for triiodothyronine than for thyroxine, in contradistinction to mammalian transthyretins. This suggests transthyretin changed from binding the "active" form of TH to binding the "precursor" form.

Aldosterone and avian species

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Aldosterone acts on the epithelia of the mammalian kidneys, gut, sweat glands and mammary glands to promote sodium conservation. Infusion of aldosterone in mammals increases the reabsorption of sodium and the secretion of potassium and hydrogen ions in the kidneys after 20-60 minutes. However, it is still not clear if the same mechanism of action is present in the avian kidneys, although aldosterone has been shown to enhance sodium and water reabsorption from the avian lower intestine. Infusion of pharmacological levels of aldosterone in pullets caused a transient decrease in sodium and chloride excretion, whereas the infusion of aldosterone at physiological levels in roosters resulted in increased plasma potassium and osmolality and increased urine flow rate, with consequent decreases in the concentrations of solute in the urine. Water deprivation resulted in a reduction of plasma aldosterone in chickens but an increase in galahs. Hypertonic saline loading reduced plasma aldosterone levels in pullets. For roosters receiving diets containing different amounts of sodium, plasma aldosterone levels were highest for birds on the low-sodium diet although water deprivation produced the greatest reduction in plasma aldosterone for the birds on the high-sodium diet. In this study, there was no correlation between plasma aldosterone concentration and sodium, potassium or ionised calcium levels in plasma. A large increase in plasma aldosterone was induced by haemorrhage in roosters. The role of aldosterone in avian species and the factors eliciting its release remain unclear.

Ventricular functioning in the bi-pyramidal heart of the eel

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The ventricle of eel hearts is unusual in that it is of a bi-pyramidal shape rather than pyramidal as in trout, or sacular or tubular as in many other fish. Presumably, the shape of the ventricle will affect the performance of the heart of a species. We were interested in the relationship between ventricular geometry and cardiac pumping strategies. In this study, dimensional changes of the ventricle of short-finned eels (*Anguilla australia*) were examined in response to varying input and output pressures and flow rates. Intraventricular pressure was recorded by inserting a catheter through the anterior face of the ventricle. The dimensional changes of the ventricle were measured from video film. The power generated by the heart was estimated from the recorded parameters. The results provide an outline on how the geometry of the eel ventricle and its mechanical arrangements affect pumping and performance. A comparison with similar results from rainbow trout, a more active fish compared to eels, allowed a descriptive analysis on how ventricular shape may be associated with cardiac performance of different fish.

Daily torpor and its energetic consequences in a small malagasy primate, *Microcebus murinus*

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The gray mouse lemur (*Microcebus murinus*), is found in the deciduous dry forests in Madagscar, and is one of only two primate genera known for their ability to enter torpor as response to low temperatures. The aim of the study was (1) to investigate the thermal and metabolic physiology of *M. murinus*, and (2) to examine the pattern of utilisation of torpor and its impact on energy budgets in free-living gray mouse lemurs. Oxygen consumption (VO_2) and body temperature (T_b) were measured in mouse lemurs that were kept in outdoor enclosures under natural ambient temperature cycles in Madagascar. Furthermore, we measured daily energy expenditure (DEE) using doubly labelled water ($D_2{}^{18}O$), and we used temperature sensitive radio collars to measure T_b and home range. Metabolic rates during daily torpor were reduced to about 20 % of normothermic mouse lemurs and T_b was regulated down to a minimum of 8 °C. The use of daily torpor by *M. murinus* significantly affected DEE in females but not in males. The DEE of torpid females was about 75% of that in normothermic females, whereas the DEE of torpid males was 5% higher than that of normothermic males. In conclusion, while daily torpor involves very low energy expenditures this need not necessarily have a significant impact on DEE.

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TBD complete with Jan

Expression of prostaglandin h synthase-2 in the endometrium and the yolk sac membrane of the tammar wallaby

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Prostaglandins are an essential part of normal parturition in tammar wallabies. A rate-limiting enzyme responsible for the synthesis of prostaglandins is prostaglandin H synthase (PGHS). A 470 bp fragment of the tammar PGHS-2 cDNA molecule was cloned using oligonucleotide primers from highly conserved regions of known PGHS-2 sequences and has 80%-90% homology with at least 7 eutherian species at the amino acid level. Southern Blotting confirmed the specificity of PCR products and showed that the gene product is expressed in the endometrium and the yolk sac placenta but not in the myometrium of the tammar reproductive tract. Tammar PGHS-2 is first expressed in the yolk sac placenta on day 19 of the 26-day pregnancy cycle. PGHS-2 is also expressed in endometrial tissues in the later stages of gestation and is higher in the gravid compared with the non-gravid uterus. Treatment of tammars with dexamethasone (glucocorticoid analogue) results in an increase in PGHS-2 expression in both placenta and the gravid endometrium. This would suggest a regulatory role for glucocorticoids in the production of prostaglandins in the tammar.

Independent effects of gravity on cranial and caudal blood pressures in aquatic and terrestrial snakes

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Changes in orientation in a gravitational field markedly alter the patterns of blood pressure and flow in animals, especially long ones such as giraffes or snakes. Vertical orientation tends to reduce blood flow and pressure in the head for two major reasons. First, the increased vertical blood column above the heart creates a gravitational hydrostatic pressure against which the heart must work. Second, expansion of dependent vessels in the lower extremities causes blood to pool there and reduces return of venous blood to the heart, thus lowering flow and pressure. For most animals it is impossible to separate these two effects, but snakes offer the possibility of bending the animal in the region of the heart and manipulating the two ends of the body independently. We studied baroregulatory responses in terrestrial pythons and aquatic acrochordid snakes by tilting only the front or rear parts and then the whole animal. Contrary to expectations, the changes in brain blood pressure during partial tilts added up to the change during full tilt, and showed that the vertical distance to the brain was about twice as important as blood pooling.

Baseline corticosterone, total and free thyroid hormones in juvenile salt water crocodiles (*Crocodylus porosus*)

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Thyroid hormones are required for the maintenance of co-ordinated growth and development of young animals and are required for basal metabolism in adults. Glucocorticoids are recognised as the main endocrine secretion associated with stress in vertebrates. The vertebrate class in which least is known about the thyroid and its secretions is the Reptilia. Within reptiles, thyroid function has been studied in lizards and snakes, less is known about tortoises and virtually nothing about the crocodilia. Also, little is known about the interaction of thyroid hormones with glucocorticoids in the Reptilia. Crocodilians are the closest living relatives of the stem reptiles from which both birds and mammals arose. Given this unique phylogenetic position, the crocodilia may provide important information concerning the evolution of function and control of hormonal systems. In this study, five 12 month old salt water crocodiles were blood sampled fortnightly for three months. Baseline levels of corticosterone and total and free thyroid hormones were measured by radioimmunoassay.

Diurnal fluctuations in the pulmonary surfactant system in two species of bat

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Pulmonary surfactant, a mixture of lipids and proteins controls the surface tension of the fluid lining the lung. It is critical for lung function and stability. The amount and composition of surfactant is influenced by physiological parameters such as metabolic rate, body temperature and ventilation. Microchiropteran bats experience large fluctuations in these parameters throughout their natural daily cycle. The activity cycle of two species of microchiropteran bats, *Chalinolobus gouldii* and *Nyctophilus geoffroyi*, was studied over a 24 h period. Bats were maintained at a constant ambient temperature (24 °C) with an 8:16 hr light-dark cycle. Diurnal changes in the amount and composition of surfactant were measured by lavaging bats at four hour intervals throughout 24 h. *C. gouldii* were most active at 2am, whereas *N. geoffroyi* experienced two peaks of activity, at 6pm and 6am. In both species the amount of surfactant increased approximately 1.5-fold when they aroused from torpor. The proportion of disaturated phospholipid remained constant throughout the 24h period. The surfactant cholesterol content increased 1.5-fold during torpor in *C. gouldii* but not in *N. geoffroyi*. The cholesterol levels of both species were up to an order of magnitude lower than other mammals. Future research should investigate how the surfactant of *C. gouldii* and *N. geoffroyi* functions with such low levels of cholesterol.

Prenatal development of the antioxidant enzyme system in the lungs of non-mammalian vertebrates

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The activity of the pulmonary antioxidant enzymes (AOE), superoxide dismutase (SOD), glutathione peroxidase (GP) and catalase increase in the final 10-15 % of gestation in mammals. It is unknown whether this increase is genetically programmed, or environmentally determined. *In utero*, a foetus experiences low pO_2 levels, so, upon birth, the infants experience a relative hyperoxia, which is associated with a large increase in the production of reactive oxygen species. *In ovo*, embryos are adapted to higher pO_2 levels and consequently do not experience such a profound relative hyperoxia. We compared the activities of catalase, SOD and GP during late development of the viviparous lizard, *Tiliqua rugosa*, and the two oviparous species, the bearded dragon, *Pogona vitticeps* and the chicken. Activity of AOE changed little throughout late development in *T. rugosa*. In oviparous species, catalase and GP activity increased in late gestation due to a genetically determined pattern. Unlike mammals, SOD activity decreased in late incubation in oviparous species. Hypoxia in chicken embryos significantly affected the activity of GP. Overall, the normoxic development pattern for the AOE appears to be genetically determined in the three species. However, under some conditions, GP and SOD are capable of responding to fluctuating environmental conditions. We propose that the level of active enzymes is genetically programmed, but there may be an "override mechanism", whereby specific enzymes can respond to the environment.

Dexamethasone and adrenaline stimulate pulmonary surfactant secretion in embryonic chicken lungs

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Pulmonary surfactant (PS), a mixture of phospholipids and proteins, secreted by alveolar type II cells, functions to reduce surface tension in the lungs of all air-breathing vertebrates. In mammals, phosphatidylcholine (PC) secretion increases in fetal lungs with advancing gestation. The stimuli for secretion are a cortisol surge, an increase in sympathetic activity and mechanical stretch of the cells. However, little is known about the factors controlling the development of the surfactant system in non-mammalian, particularly egg-laying, vertebrates. Here, we examined the effects of dexamethasone, adrenaline and acetylcholine on PC secretion in the developing chicken lung. As dexamethasone is believed to act on type II cells through paracrine factors released from interstitial fibroblasts, type II cells and lung fibroblasts were isolated and co-cultured from chickens following 16, 18 and 20 days of incubation and from hatchlings. Basal and stimulated secretion were most pronounced at day 18, just prior to the onset of air breathing. Adrenaline stimulated PC secretion by greater than 100 % at all stages, whereas dexamethasone stimulated secretion of PC by 50 % or more at day 18. Acetylcholine had no effect at any stage. Therefore, it appears that similar stimuli control PS secretion in mammalian and avian lung development. This is despite the absence of maternal influences in the chicken embryo and vastly different birthing strategies between the two groups.

Stress and tuna flesh

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The effect of harvest stress on Southern bluefin tuna (*Thunnus maccoyii*) was investigated. Fish ..cted to simulated harvest stress were compared to unstressed controls. Commercial harvest ..ds (ie line caught vs net harvest) were also compared. Blood and muscle tissue were ..nected at death and during ice storage up to 24 h post-mortem. At death plasma cortisol, lactate and pH, and muscle, lactate, glycogen and pH were significantly different in the treatment groups of the respective experiments. These results are discussed in terms of post-mortem changes and implications for tuna flesh quality. This work is part of ongoing research to modulate the flesh quality characteristics of farm-raised tuna through capture-harvesting practices.

An overview of the natriuretic peptide receptor system in the gills of fishes

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Natriuretic peptide (NP) hormones function in blood volume regulation in mammals and are present in all fish classes. In fishes, NPs also appear to affect blood volume via vascular effects and ionic regulation. The heart is the major site of secretion of NPs and, because the circulation of fishes is in series, all the blood passes though the gills before entering the systemic circulation. One of the puzzles about the function of NPs in fishes is that the gills have a large population of NP receptors that effectively clear the majority of circulating NPs before they reach other target organs such as the kidneys. The gill NP receptors are located at different sites depending on species but, apart from a small population of the biologically active guanylyl cyclase receptors, the majority of them are not known to have biological function except for clearance. Research in mammals has indicated that clearance receptors may be associated with decreases in the second messenger, cAMP. Recently, we have examined whether NPs affect cAMP production in fish gills. Forskolin-stimulated cAMP production was slightly inhibited by atrial NP but not C-ANF, a specific clearance receptor ligand, suggesting that the inhibition is not mediated through the clearance receptors.

Physiological effects of capture and transportation in the coral trout, *Plectropomus leopardus*

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Coral trout, *Plectropomus leopardus*, are an important live export and aquaculture species. In response to capture by hook and line, *P. leopardus* exhibited a typical stress response involving a significant elevation of plasma cortisol, glucose, lactate, osmolality and haematocrit (Hct). Following a 24 h recovery period these parameters returned to resting levels. Fish sampled immediately upon arrival after air-freighting showed elevated plasma cortisol in comparison to the baseline levels found in wild caught fish. However, the concentrations present were lower than the maximum levels reached following hooking. The rise in plasma glucose to near maximal post-hooking levels suggests that the fish were stressed. This was not a hypoxic stress as plasma lactate was not significantly elevated from baseline levels. Differences in osmolality were confounded by different external salinities. Fish hand-netted from holding tanks and exposed to air for either 0, 2, 4 or 6 minutes also showed signs of stress including elevated cortisol levels. Hct was highest in the 6 min treatment. Hct is elevated as a means of increasing the blood oxygen carrying capacity. Osmolality, glucose and lactate were all highest in the 4 minute treatment group. Reasons for subsequent decline were not clear.

Adrenocortical responses to capture in northern tuatara (*Sphenodon punctatus punctatus*) from a rodent-free and a rodent-inhabited island

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Recent studies on reptiles have considered basal levels of plasma corticosterone, and the adrenocortical response (magnitude/duration of elevation in corticosterone in response to capture), as indicators of chronic stress. We examined these in two populations of tuatara, the only surviving sphenodontian reptile. We hypothesised that tuatara (*Sphenodon punctatus punctatus*) on Coppermine Island, where introduced rodents, *Rattus exulans*, are present (potential predator/competitor) would have a higher adrenocortical response than those on Green Island (rat-free). Blood samples taken in February 1997 from adult tuatara at 0, 3 and 18 h post-capture, were analysed for plasma concentrations of CORT, testosterone, progesterone and glucose. Although initial CORT levels were slightly higher on Green Island than on Coppermine Island ($p<0.02$), Coppermine Island tuatara of both sexes had significantly higher CORT levels than Green Island tuatara at both 3 h and 18 h ($p<0.0001$). These results confirm that wild populations of reptiles can differ in their adrenocortical responses, and that basal levels of CORT do not necessarily match the direction of differences in the adrenocortical response. If the higher adrenocortical response in Coppermine Island tuatara was caused by the presence of introduced rats, the difference should eventually disappear following the eradication of rats on this island.

Birth in the brushtail possum, *Trichosurus vulpecula* (Marsupialia:Phalangeridae)

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Birth has now been observed and described in a number of marsupials. However the process is still not fully understood. Birth and manipulation of newborn young in the brushtail possum has been filmed to provide a greater understanding of the mechanisms involved. Prior to birth, females began to lick the pouch and urogenital sinus vigorously. Each female assumed the birth position, sitting on the base of the tail, with tail extended forward between the flexed hind legs. The young crawl upwards and reach the pouch within 2 to 3 minutes and have attached to the teat within 5 minutes. Mothers were anaesthetised immediately following parturition and the young removed from the teat and placed 3 cm above, below or to the side of the pouch on the mother's fur. In each instance, the young were able to find the pouch and attach to the teat. If the young was placed more than 3 cm above the pouch, it crawled upwards towards the mother's head. Thus it appears in the possum that the ability to sense gravity and olfaction play a role in allowing the newborn to locate the teat.

Locomotion energetics of the brush-tailed bettong

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Hopping kangaroos and wallabies show rates of oxygen consumption that do not change over large ranges of speeds. Most early researchers did not find similar oxygen consumption patterns in small hopping mammals. However, the prevalence of small hopping mammals suggests that hopping may confer some advantage even to small animals. Juveniles of the larger kangaroo species are able to keep up with their parents when hopping, and large kangaroos evolved from smaller ancestors. It is possible that energy efficient locomotion had already evolved in small ancestral hoppers.

The brush-tailed bettong is a small (mass 1 kg) potoroid marsupial which is an obligate hopper at high speeds. We have examined both locomotion energetics and gait patterns in this species. Our results suggest that hopping at high speeds is energetically cheaper than the cost of running in a similarly sized quadruped. In addition, the brush-tailed bettong appears to be comparatively more efficient than the red kangaroo at these higher speeds.

Characterization of myosin heavy chain isoforms of limb muscles of macropodids

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Myosin heavy chain (MyHC) isoforms in limb muscles of marsupial mammals have not been characterised. We used SDS gel electrophoresis, Western blotting and immunohistochemistry to investigate limb muscle MyHCs in 4 species of macropodids using highly specific monoclonal antibodies (mabs) against the 4 eutherian MyHCs found in adult limb muscles. SDS gels of macropodid limb muscles showed a total of 4 bands reminiscent of the eutherian slow (fastest migrating), IIB, IIX and IIA (slowest migrating) isoforms. Western blots of these bands using the mabs confirmed these correspondences. These mabs stained 4 distinct types of fibres in macropodid tibialis anterior muscles. The immunoreactivity and size of these fibres showed that they corresponded to type I (slow), fast IIA (small diameter), IIX (medium diameter) and IIB (large diameter) fibres found in eutherian muscles. Our data suggest that the 4 marsupial MyHCs are homologous to their eutherian counterparts. These findings suggest that genes encoding these MyHCs are shared by the common ancestor of eutherian and marsupial mammals, and that these genes have diverged from each other at least 140 million years ago. The predominance of fatigue resistant IIA and IIX fibres in the gastrocnemius is an appropriate adaptation for sustained hopping.

ABSTRACTS OF POSTER PRESENTATIONS

Faecal testosterone concentrations may not be useful for monitoring reproductive cycles in blue-tongued lizards

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Faecal steroid monitoring is a non-invasive technique that may be suitable for following reproductive cycles in both captive and free-range situations. The only published work on reptiles is on the Galapagos and other related tortoise species. In the blue-tongued lizard, *Tiliqua nigrolutea*, we were able to measure faecal testosterone concentrations in males, although an interfering substance in the extract necessitated additional clean-up steps compared with published extraction techniques. Faecal testosterone concentrations ranged from 500 ng g^{-1} to 1500 ng g^{-1}, with a significant difference ($P < 0.05$) in concentrations between mating and quiescent males. However, the profile of faecal testosterone through the reproductive cycle did not parallel the plasma testosterone profile. Further work is needed to indicate the possibilities for successful utilisation of faecal steroid monitoring in reptiles.

Crural Muscle function in the pelvic limb of the turkey, *Meleagris gallapavo*

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Strain gauges were bonded to mineralised tendons in the crus of turkeys were used to determine force production in individual muscles throughout the gait cycle. Simultaneous strain, electromyographic and video recordings were made as birds 'walked or ran' on a motorised treadmill, at speeds of between 0.25 and 2.8 m/s. Duty factor decreased continuously and linearly with increasing speed. Peak tendon strain (≡ muscle force) increased with increasing speed for all muscles examined. However, the shape of the force-time curves for muscles varied markedly - (a) between muscles, and (b) with increasing speed within the same muscle. The results revealed a variability of function of individual muscles within a gait. The nature of the force-time curves for *m. fibularis longus*, *m. tibialis cranialis* and *m. gastrocnemius* indicated that complex muscle function occurs during locomotion. The curve for *m. tibialis cranialis* generated significant force during the swing phase, being inactive during stance. Maximum forces for all muscles, measured during the highest running speeds, were significantly lower than the estimated peak isometric muscle force capacities calculated from muscle mass and fibre length measurements. A detailed understanding of muscle function during locomotion can be gained by this *in vivo* experimental approach.

An alternative to 17β-oestradiol in a viviparous reptile?

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17ß-Oestradiol (E2) is the predominant oestrogen in most vertebrates, including reptiles. However, an additional oestrogen, oestrone (E1) has been identified in plasma or from gonadal incubations in several reptiles. In the snake *Thamnophis sirtalis parietalis*, two alternative oestrogens, 6α- and 6ß-hydroxyoestradiol are present in high concentrations in plasma (Whittier and Hess, 1992). We have examined plasma concentrations, and gonadal and peripheral production of oestrogens in male and female blue-tongued lizards, *Tiliqua nigrolutea*. 17ß - Oestradiol was not detected in incubations of gonadal tissue with pregnenolone (P5), and may only be present in extremely low concentrations in plasma, (Lance, pers, comm) despite assay results suggesting a clear and physiologically meaningful annual pattern of circulating oestrogen concentrations. We propose the presence of an alternative to E2 as the predominant oestrogen in *T. nigrolutea*.

Whittier J.M. and Hess D.L. (1992). The occurrence of 6-substituted estradiol 17ß in the plasma of female garter snakes (*Thamnophis sirtalis parietalis*). Adv. Comp. Endocrinol. 1 77-82.

Effect of thermoperiodic acclimation on rhythms of thermal selection in lizards

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It is well known that thermoregulatory parameters of reptiles can be modified by photothermal acclimation. This study examines how long-term acclimation influences rhythms of thermal selection in the scincid lizard, *Tiliqua rugosa*. These lizards undergo a daily voluntary hypothermia whereby they seek out lower temperatures at night in an artificially imposed light cycle in a laboratory thermal gradient. In the first experiment we tested the hypothesis that acclimation to 6 h cold pulses of 15 °C in an otherwise 33 °C environment and 12L:12D photocycle could influence the nighttime hypothermia, depending upon the phase relationship between the thermocycle and the photocycle. Groups of lizards were subjected to a cold pulse around dawn, midday, dusk or midnight for 6-7 weeks in autumn after which they were tested in a thermal gradient for 72 h in a 12L:12D photocycle. The daytime temperature selected by all four groups was unaffected by the acclimation treatment, but the nighttime temperature varied depending upon the phase of the cold pulse. The highest temperature was with a pulse delivered at dusk, the lowest with a pulse delivered at midnight, and the dawn and midday pulses having intermediate effects. In a second experiment, we tested the effect of 6 h cold pulses in constant light (LL) to determine whether the interaction of light and temperature cycles influenced the nighttime selected temperature in experiment 1. Four groups of lizards were acclimated for 6-7 weeks in autumn to cold pulses delivered at the same times as in the previous experiment, but in LL, and then tested for 72 h in a thermal gradient in a 12L:12D photocycle. The nighttime selected temperature did not differ significantly among the four treatment groups. These experiments indicate that light and temperature cycles interact with a circadian clock to affect seasonal changes in nighttime body temperatures of these lizards.

Spontaneous torpor in captive dusky woodswallows (*Artamus cyanopterus*)

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Because information regarding torpor in Australian birds is limited we investigated whether dusky woodswallows are heterothermic. They appeared likely candidates for use of torpor since they are small (~35g) and eat primarily aerial insects whose abundance fluctuates and whose capture is energetically expensive. We caught two fledglings (approx 13 days old) in January 1998, hand-reared them and kept them in an outdoor aviary exposed to natural photoperiod and temperature fluctuations. Tenebrio larvae and other arthropods were provided ad libitum. Temperature-sensitive transmitters were implanted under general anaesthesia. From 20 April to 22 June body temperature (T_b) was recorded every 6 minutes. Daily fluctuations of T_b were pronounced: normothermic T_b during the photophase was about 39°C, decreased significantly at dusk to a minimum of 29.2°C, remained low throughout the scotophase (about 12 hrs), then increased to normothermic T_b at dawn. Birds entered torpor (T_b < 35°C) on 42/48 nights measured and maintained body condition during the study period. Torpor was never observed during the photophase. Our study provides the first quantitative information of spontaneous torpor in an Australian passerine. It shows that even though Australia is typically thought of as a warm continent, at least some birds regularly use torpor for energy conservation even when food is readily available.

Melatonin administration and reproduction in the marsupial *Antechinus stuartii*

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Antechinus stuartii is a marsupial with a highly synchronised reproductive life history, which culminates in the death of all males from a combination of raised circulating androgens and cortisol. The regulation of the synchronous life history appears to be controlled by photoperiodic change over the year. Oral administration of the pineal hormone melatonin from the winter solstice (late June, when photoperiod begins to increase) has been found to affect the reproductive response of *A. stuartii* to increasing rate of change of photoperiod. Oestrus in the females from the first week of August (controls) to an earlier time of mid-July and the consequent pouch changes associated with pregnancy and pseudopregnancy were also shifted by the same length of time. The post-mating decline and consequent death in the males were also accelerated. In the present study melatonin was orally administered from the autumnal equinox (late March) in an effort to "flood" the animals with melatonin and thus determine whether pineal hormone actions were important in establishing synchrony. It was found that for melatonin treated females reproductive activities were desynchronised, with oestrus occurring over an eight-week period, compared to two weeks for control females. Male maturity and postmating decline was also not as synchronised as for control males. It thus appears that since melatonin administration affects reproductive timing in *Antechinus stuartii*, melatonin action, and therefore pineal activities, are important in the regulation of reproduction in this marsupial.

Effect of sodium depletion on the concentration of cAMP and the activity of Na^+/K^+-ATPase in the gills of Crayfish *Cherax destructor*

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The roles of neuroendocrine factors and second messenger systems in the control of osmoregulatory processes has been investigated in a variety of decapod crustaceans (1). In hyperosmotically regulating crabs ion influx across the gills is stimulated by dopamine and a rise in the intracellular levels of cAMP (2,3). The aim of the present study was to determine whether cAMP is also used as a second messenger responsible for the activation of ion transport in freshwater decapods, notably the gills of the freshwater crayfish *Cherax destructor*. Two groups of crayfish were used. One group was acclimated to 25% seawater (~125 mmol/L Na) for one week in order to minimise sodium uptake while animals in the second group were depleted of sodium by keeping them in Na-free artificial tap water (ATW) for one week. The animals were then killed and the levels of cAMP and activities of Na^+/K^+-ATPase in their gills were determined. The concentration of cAMP was significantly elevated ($p<0.001$) in the gills of sodium-depleted crayfish (289.30E.91 pmol/g tissue (n=9)) compared to the value for the gills of sodium replete animals (143.00F.56 pmol/g tissue (n=10)). The activity of Na^+/K^+-ATPase in the gills of sodium depleted crayfish (1.111.141 U/g tissue (n=9)) was also significantly elevated ($p<0.001$) compared to the activity in sodium replete animals (0.529.148 U/g tissue (n=10)). Sodium depletion results in an elevation of activity of the transport enzyme and a concomitant increase in intracellular levels of cAMP. It is probable that in freshwater crayfish, as in euryhaline crabs, cAMP is the intracellular messenger utilised to control the level of branchial ion transport and that this is effected at least in part by the modulation of the activity of Na^+/K^+-ATPase. Extension of this work will focus on the time course of the elevation of cAMP and the bioamines that may be involved in the regulatory processes.

Handling stress and its effects on ventilation in two reptiles

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The impact that we, as scientists, have on the animals that we use for experimentation is often underestimated, but has far reaching consequences on not only animals' welfare and health, but also on the relevance and value of the data collected. The mammalian stress response is well documented and includes increases in heart rate, ventilation and plasma corticosteroids. The response of reptiles to stressors, however, remains relatively understudied. Handling is a common and often unavoidable stressor placed on animals used in research, however studies on the effects of handling in reptiles have been limited to neuroendocrine and behavioural responses. In this study the effect of handling on ventilation was measured in two reptiles (*Chelodina longicollis* and *Physignathus lesueurii*). Lizards increased minute ventilation up to 17 fold immediately after handling, while the tortoises showed up to a 13 fold increase. In both species, minute ventilation, breathing frequency, tidal volume and the duration of apnoea returned to control values after one hour. These results suggest that a minimum one hour equilibration period after handling should be allowed before conducting experiments on ventilation in these species.

This total pigment went to market: Individual variation in myoglobin content in light muscle of farmed juvenile southern bluefin tuna *Thunnus maccoyii* (Castlenau)

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Tuna 'light' muscle attains premium prices as sashimi because oxy-myoglobin gives the flesh an attractive red colouration. The published myoglobin contents of particular tuna species often vary by up to two fold but it isn't known whether this variation influences the flesh colour. We measured total pigment (myoglobin) content of light muscle of farmed juvenile (~20kg) southern bluefin tuna *Thunnus maccoyii* during trials of manufactured feeds being run in 1998 and 1999 at Port Lincoln in South Australia. We compared these to colour measurements of the flesh samples obtained using a Minolta CR-300 colour meter. Pigment content in the light muscle of southern bluefin tuna ranged from 0.98 to 2.85 mg/g wet weight, similar to ranges reported for other tuna species, but this variation in total pigment content explained little of the variation in colour of the flesh. The cause of the variation between fish is presently unclear. While, pigment content varies between tuna species on a wet weight basis, it probably also varies between discreet 'cuts' of flesh drawn from the same fish. Other tissue components, for example fat droplets, lack myoglobin, and their proportional contribution to the sample wet weight may influence the measured pigment content.

Switching off the thermostat: thermoregulation by eastern skunk cabbage (*Symplocarpus foetidus*)

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The protogynous inflorescences of eastern skunk cabbage, *Symplocarpus foetidus*, are thermogenic and regulate spadix temperature (T_s) well above ambient temperature (T_a). Continuous records of oxygen consumption, carbon dioxide production, and temperatures of plants were made at a field site in Canada. At T_a between 3 - 24°C, T_s ranged between 16 - 26°C, and the warmest inflorescences were those in the receptive female or early pollen-bearing stages. Respiratory rates of the 2-g spadices increased with declining T_a, and reached a maximum of 0.73 ml/min, equivalent to 0.26 W of heat production. At T_a below 3°C, several inflorescences failed to maintain high T_s and abruptly switched T_s to near freezing. Some froze when T_a dropped to about -10°C. Those that did not freeze could quickly switch to the warm state if T_a rose above about 3°C. Switching was related to the balance between heat production and heat loss that tended to produce stable equilibria at either high or low T_s. Switching between warm and cool states resulted in a bimodal distribution of T_s in the field. A respiratory quotient of 1.0 showed that carbohydrate was the substrate for thermogenesis, and bomb calorimetry of florets confirmed that energy was imported from the root.

Why do lobsters recover oxygen uptake during prolonged air exposure?

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On emersion, the oxygen consumption of *Jasus edwardsii* is observed to decrease initially but progressively recovers during extended air exposure. The lobsters also experience a temporary bradycardia. We tested hypotheses that these changes in oxygen consumption are due to one, or a combination, of the following:

1. Metabolic regulation - the metabolic rate of the tissues decreases in air and later increases and this determines oxygen uptake at the gills;
2. Perfusion limitation - reduced gill blood flow initially limits gas exchange and this recovers with the increase in heart rate;
3. Diffusion limitation - water adhering to the gills initially reduces their conductance which improves as they dry;
4. Haemocyanin-oxygen binding - the affinity for oxygen of haemocyanin increases as emersion proceeds and compensates for one of the above.

Blood lactate, cardiac output, the diffusing capacity of the gills, and haemocyanin-oxygen affinity all increased during emersion. It is concluded that the increased Mo_2 during emersion is due, mainly, to the increased diffusing capacity of the gills as they dry and, partly, to an increase in the affinity of haemocyanin. The increase in oxygen affinity may be caused by the accumulation of lactate in the haemolymph. An unexplained observation is that a second immersion/emersion cycle does not cause the same decrease in Mo_2 as the first.

The physiological effects of ectoparasite infection of coral trout, *Plectropomus leopardus*

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Coral trout are the subject of an important and expanding live-fish export industry and are a strong candidate for tropical marine aquaculture. Parasitic infection can adversely affect cultured finfish productivity. The project aimed to determine whether a causal relationship exists between ectoparasite load and physiological status. The physiological status was determined from assaying blood samples taken within 60 s of hooking. The results, indicative of baseline stress, were compared to the longer term stress response due to hooking, handling and captivity. Ectoparasite number was positively correlated with fish size. Copepods cause attachment trauma to the gills (*Dissonus manteri*, 4-165/fish; *Hatschekia plectropomi*, 35-479/fish), and the upper palate (*Lepeophtheirus plectropomi*, 2-145/fish). *Dentigryps litus* (1-8/fish) move freely over the body surface. No significant correlation existed between ectoparasite load and physiological stress. Glucose concentrations of 15.7 mM occurred 2 h post-hooking, whereas initial samples never exceeded 2.6 mM. Equivalent values for osmolality were 494 and 434 mOsm/l, and for cortisol, 50 nm and 0.4 nm indicating that levels of ectoparasite infection on wild fish were insufficient to cause significant physiological disturbance to the host fish.

Pelvic limb anatomy of the red kangaroo and brush-tailed bettong

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Hopping kangaroos and wallabies show rates of oxygen consumption that do not change over large ranges of speeds (Dawson and Taylor, 1973; Baudinette *et al.*, 1992). Recently, a similar pattern was observed for hopping Brush-tailed Bettongs (Webster and Dawson, unpublished). Although the relatively low cost of locomotion in kangaroos has not yet been fully explained, it appears to be associated with both storage of elastic strain energy and powerful locomotory muscles. We have examined the pelvic limbs of the Red Kangaroo (mass 25-80 kg) and the Brush-tailed Bettong (mass 1 kg). In both species, probable areas of elastic energy storage were the large, thick tendons associated with the gastrocnemius and caudofemoralis muscles. Muscles used for power production during hopping were the biceps femoris, the quadriceps group, caudofemoralis, adductor, gluteal group and gastrocnemius. There were some small differences in relative size of these muscles between the two species.

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The effect of glucoprivation on thermal physiology of *Sminthopsis macroura*: torpor or hypothermia?

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Glucose availability may play an important role in triggering torpor onset because 2-deoxy-D-glucose (2DG), a glucose inhibitor, induces a torpor-like state in heterothermic placental mammals. We tested whether glucose deprivation by 2DG shows the same effect in the carnivorous marsupial *S. macroura* (25g) and whether the 2DG-induced state differs from natural torpor. We administered three doses of 2DG early in the morning and measured body temperature (T_b) and metabolic rate to quantify physiological variables. Incidence of torpor was highest for natural torpor (80%). Similar effects were observed at the highest 2DG dose, but the lower doses were less effective. Maximum rate of entry (approx. 0.2 °C/min) was similar for natural torpor and the highest 2DG dose; the low 2DG dose resulted in slower entry rates. Mean natural torpor was more than twice as long than the 2DG-induced state, but dose did not effect its duration. Minimum T_b was significantly lower and arousal from natural torpor was about two-three times faster than for the 2DG-induced state. Our study shows that 2DG induces a torpor-like state in *S. macroura*. However, physiological variables differ greatly from those of natural torpor, suggesting that 2DG causes reversible hypothermia rather than torpor.

Locatisation of follistatin in testes of rat and brushtailed possum (*Trichosurus vulpecula*)

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There is increasing evidence that activins and inhibins have local effects within the testes. Follistatin binds both activins and inhibins, thus neutralizing most of their actions. The follistatin gene is highly conserved across species and encodes a single-chain protein which exits in at least two alternatively spliced variants, each with varying degrees of glycosylation. Follistatin is expressed in many tissues, including the pituitary, ovary and testis. In this study, we examined the localisation of follistatin in testes from the rat and brushtailed possum using immunohistochemistry.

An antiserum raised in chickens against a synthetic peptide corresponding to amino acids 121-133 of follistatin was used as primary antibody. Testes of adult rats and possums were fixed in Bouin's fluid, and sections were subjected to antigen retrieval by microwaving, followed by incubation with primary antibodies, biotinylated anti-chicken antibodies and streptavidin-alkaline phosphatase successively. Colour reaction was developed by BCIP/NBT. Normal chicken serum was used as negative controls.

The antiserum stained Leydig cells but not Sertoli cells in the rat testes, while in the possums, Sertoli cells were intensively stained, and Leydig cells were weekly stained. No staining was observed in spermatogonia and spermatocytes either in possum or in rat testes. The results indicate that the role of follistatin may be different between species, or alternatively, each species may express different forms of follistatin which are not detected by this antibody.

Effects of oxidative insult on red blood cells from high and low GSH sheep

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Incubation of low-glutathione (GSH) red blood cells (RBCs) and GSH-depleted sheep RBCs with t-butyl hydroperoxide (tBHP, 3mM) for 10 min caused the formation of TBARS, oxidation of haemoglobin and degradation of membrane proteins. By contrast, high-GSH RBCs (normal RBCs) did not undergo the degradation of membrane proteins within the first 10 min. Dithiothreitol (DTT) was highly effective in preventing the tBHP-mediated oxidation of haemoglobin, the formation of TBARS and the degradation of membrane proteins in both normal RBCs and low-GSH RBCs. However, DTT did not provide protection in GSH-depleted RBCs or normal RBCs in the presence of 1.5 mM mercaptosuccinate, a potent inhibitor of GSH peroxidase (GSHPx). The ability of GSH to prevent the oxidation of haemoglobin and the degradation of membrane proteins was abolished in the presence of mercaptosuccinate. These results indicated that the function of DTT involved a GSH-dependent mechanism. Both GSH and GSHPx played key roles in this enzymatic system. If insufficient GSH was provided or alternately if GSHPx was inhibited, the enzymatic system was rendered ineffective. In the light of the complete protection of RBCs against oxidation induced by tBHP in the presence of DTT or GSH, the main effect of GSH/GSHPx appears to be directly as a tBHP scavenger. The functions of four well-known antioxidants, Butylated hydroxytoluene, ascorbate, a-tocopherol and desferrioxamine were also tested in this study to cast further light on the role of free radical scavenging in protection from tBHP mediated free radical insult.

Different mechanism for the glycerol induced haemolysis of human and sheep red blood cells

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To further the understanding of the mechanism of haemolysis in glycerol media, the effects of pH on the rate of haemolysis in human and sheep red blood cells (RBCs) were studied. The rate of haemolysis was observed in human and sheep RBCs from pH 5.8 to pH 10.0. Over the whole pH range, the rate of haemolysis decreased with increase of pH in sheep RBCs. By contrast, the rate of haemolysis increased from pH 5.8 to pH 6.4 and decreased above pH 6.4 in human RBCs. The rate of haemolysis in sheep RBCs at pH 5.8 and pH 6.4 and in human RBCs at pH 5.8 exhibited temperature-dependence. However, the rate of haemolysis in human RBCs at pH 6.8 was not influenced by temperature. Cu^{2+} partially inhibited the rate of haemolysis in human RBCs but not in sheep RBCs. These results indicate that the different effects of pH on the rate of haemolysis in human and sheep RBCs may be due to inhibition of glycerol permeability by H+ in human RBCs but not in sheep RBCs. Our results do not support a previous suggestion that the effect of pH on the rate of haemolysis was not due to glycerol permeability, but to a process involving band 3. We did not observe the complete inhibition of haemolysis by the classical band 3 inhibitor, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). Another band 3 inhibitor 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS) showed only weak inhibition. Phenylgloxal (PG),another band 3 inhibitor had no effect whatever on the rate of haemolysis. These results indicate that the anion pathway of band 3 is not the preferred route of transport of glycerol. Since DIDS showed a similar inhibition of the rate of haemolysis in both human RBCs and in sheep RBCs, the inhibitory effect of DIDS could not be due solely to its inhibition of AQP3 (Aquaporin 3), a major pathway for glycerol transport in human RBCs, only.

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