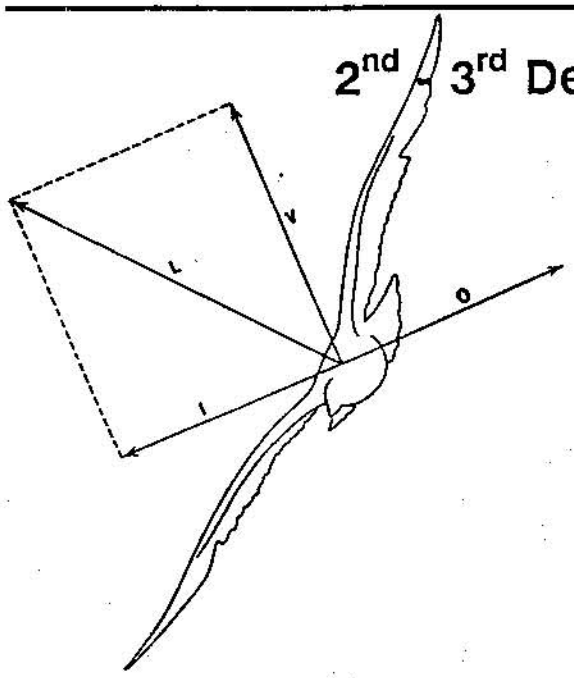
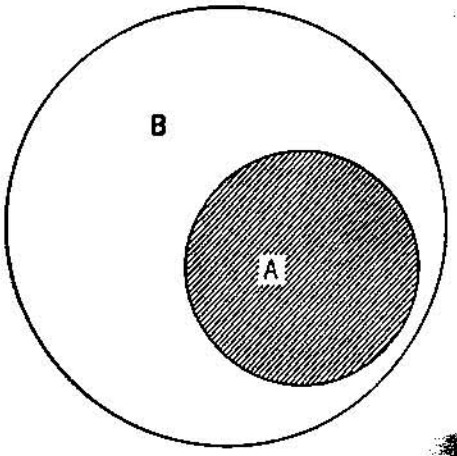
F. GEISER

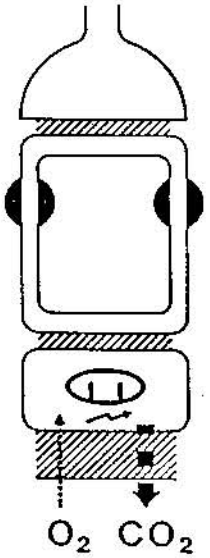
SIXTH
COMPARATIVE PHYSIOLOGISTS MEETING

2nd 3rd December 1989



PROGRAM
&
ABSTRACTS





School of Biological Sciences
Flinders University of South Australia

Organisers: R.V. Baudinette & P.B. Frappell

SIXTH
COMPARATIVE PHYSIOLOGISTS MEETING
2nd - 3rd December 1989

Saturday

Registration	0830
Opening Comments & Housekeeping Matters	0930

Session 1: OXYGEN — Chair: **David Jones**

Title	Author	Time
Cardiovascular Responses to Hypoxia in the Tuna.	P. Bushnell R. Brill	0940
Inter-atrial Septal Changes in the Perinatal Marsupial Heart.	S. Runciman B.J. Gannon R.V. Baudinette	1000
Is the Size of the Joey Limited by Constraints imposed on the O_2 Cascade?	P.B. Frappell	1020
Interaction of Organic Phosphates with Sheep Haemoglobin.	R.A.B. Holland J.F. Hallam E.A. Tibben	1040

Session 2: OXYGEN — Chair: **Russ Baudinette**

Structural Changes in the Tammar Wallaby Lung from Birth to Adult.	J. Power	1120
Cardiovascular Dynamics of the Alligator.	D. Jones G. Shelton	1140
Respiratory Adaptations to Emersion in the Mudcrab.	G. Varley	1200
The Role of Gills and Lungs in Air-breathing Crabs.	C. Farrelly P. Greenaway	1220

Picnic Lunch
Anchor Courtyard
All provided

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COMPARATIVE PHYSIOLOGISTS MEETING
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Saturday

Session 3: DEVELOPMENT	Chair: **Bren Gannon**	
Patterns of Metabolism in Embryonic Reptiles.	M.B. Thompson	1400
Respiration in the Developing Port Jackson Shark Embryo.	K. Messner	1420
Respiration in Terrestrial Frog Eggs.	R. Seymour	1440
Growth Hormone Secretion in the Tammar Through Development.	E. Bugledich P.A. Janssens L.A. Hinds	1500
Release of the Embryo from Diapause Near the End of Pouch Suckling in *Bettongia penicillata*.	M.J. Smith	1520

Session 4: FOOD AND ENERGY	Chair: **Roger Seymour**	
Heat Production in the Echidna.	S.C. Nicol N.A. Andersen U. Mesch	1600
Blood Gases and Body Temperature in the Echidna.	N.A. Anderson S.C. Nicol U. Mesch	1620
Periodic Arousing During Hibernation.	F. Geiser	1640

General Discussion of Today's Papers: P. Bushnell & P. Frappell

University Club
Drinks commence 1730 hrs

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Sunday

Session 5: WATER	**Chair: Peter Janssens**	
Effect of Salt Load on Renal Function in Feral Chickens.	J. Roberts	1000
The Influence of Dietary Phosphate on pH and Buffering Capacity of the Haemolymph in the Cricket.	P. Cooper R.L. Guy	1020
Phenotypic Inhibition of the Renin-Angiotensin System in Mice Preceded Extensive Expression of Salivary Gland Renin.	S. Skinner D. Weaver L. Walker M. Sangster	1040

Session 6: Regulation	**Chair: Sandy Skinner**	
Osmotic Regulation in the Anomuran Land Crab *Birgus latro*.	P. Greenaway	1120
Intracellular pH Buffering in Hagfish Muscle.	J. Baldwin	1140
Blood Brain Barrier: A Marsupial Alternative to the Eutherian Solution.	C.J. Carati G.H. White B.J. Gannon	1200
Giant Dinosaurs with Long Necks: A Model to Determine the Most Efficient Lung and Metabolism.	C.B. Daniels J. Pratt	1220

Biology BBQ Area
All provided

SIXTH
COMPARATIVE PHYSIOLOGISTS MEETING
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Sunday

Session 7: MOVEMENT & INTEGRATION	Chair: **John Baldwin**	
Cardiac and Locomotor Coupling: A Human But Not a Macropod Solution.	R.V. Baudinette B.J. Gannon	1400
Chemical Coding of Parasympathetic Neurons of the Toad, *Bufo marinus*.	P. Davies H. Cousins G. Campbell	1420
Neuropeptides in the Sympathetic Neurons of Lizards: Correlations with Body Size and Thermoregulatory Capacity?	I. Gibbins J. Morris S. Holmgren	1440
Adrenergic Regulation of Hepatic Gluconeogenesis in Vertebrates.	P. Janssens M. Giuliano J. Grigg	1500

Closing Comments, Site of Next Meeting
& Seasons Greetings from
Gordon Grigg
Terry Dawson
William Runciman

Intracellular pH Buffering of Hagfish Muscle

John Baldwin, Department of Botany and Zoology,
Monash University, Clayton, Vic. 3168

Anaerobic muscle work is accompanied by proton generating processes which have the potential to significantly alter intracellular pH. In the muscles of vertebrates the imidazole group of histidine (present as the free amino acid, dipeptides or protein histidine), and to a lesser extent phosphates, function to buffer pH over the physiological range. Consequently, correlations exist between the pH buffering capacity of a muscle and its potential for anaerobic work.

Intracellular pH buffering capacity of hagfish (Eptatretus cirrhatus) tongue muscle is among the highest reported for any vertebrate muscle. Over 80 per cent of the buffering capacity of tongue and myotome muscle is due to components other than proteins and phosphate. The muscles have less than 0.5 μmol g^{-1} wet weight of L-histidine, and lack the histidine-containing dipeptides anserine, carnosine and ophidine. Instead, they contain an unidentified low molecular weight acid soluble compound (myxinine) to which the high pH buffering capacity of tongue muscle can be attributed.

CARDIAC AND LOCOMOTOR COUPLING: A HUMAN BUT NOT A MACROPOD SOLUTION

R.V.Baudinette and B.J. Gannon
Schools of Biological Sciences and Medicine
Flinders University

Coupling of respiratory and locomotor rhythms has been well established in a range of mammals (see the review of Bramble, D.M.: Amer Zool. 29: 303-17. 1989) and forms the basis of a visceral respiratory pump in macropods (Baudinette, R.V. et al: J expt Biol 129: 251-63. 1987).

Kangaroos and wallabies have a conical profile and, when hopping, opposing sets of muscles are simultaneously in an active state: both factors leading to a large reflected component of the arterial pressure pulse. We hypothesised that cardiac work would be minimised and blood flow to active muscle maximised if peak arterial pressure occurred at the time of lowest intramuscular pressure; i.e. when the animal was in the suspended phase of the step cycle. This would require that heart rate was entrained with limb movement, perhaps with an inbuilt phase lag to accommodate the time taken for the pulse wave to reach the hind limbs.

From recent results obtained with macropods it is clear that this hypothesis cannot be sustained. We have hopped tammar wallabies on a treadmill while they were instrumented with accelerometers and transducer-tipped catheters anchored in the abdominal aorta. There was no frequency or phase relationship found between cardiac and limb frequencies.

Pressure measurements taken during the experiments show that peak arterial pressures seldom exceed 210 mm Hg, well within the range seen in other mammals and birds. How then are the potentially damaging pressure excursions resulting from synchronous limb movement relieved in macropods? The answer seems to lie in the physical properties of the aorta. The structure of the wall has a higher proportion of elastin than in the rabbit and its static compliance is about five times as great.

This situation contrasts with that seen in man where, in a recent study (Kirby. R.L. et al. J Appl Physiol 66: 323-329. 1989), cardiac coupling was observed and was suggested to be a normal physiological phenomenon.

It appears therefore that some animals may have solved the problem of high intra-muscular and aortic pressures during exercise by the entrainment of limbs and the heart. In macropods, the group with the simplest form of gait, the solution seems to lie in a change in the properties of the main arterial vessel.

GROWTH HORMONE SECRETION IN THE TAMMAR THROUGH DEVELOPMENT

E-M. A. Bugledich, P.A. Janssens & L.A. Hinds*,
Dept of Zoology, ANU, & *CSIRO Wildlife & Ecology, Canberra

Physical growth and developmental changes in the pattern of plasma growth hormone (GH) secretion and concentration were determined for 2 groups of tammars: Group 1 was born late January-early February 1987 (n=16) and Group 2 was born 2nd June 1987 (n=8).

GH secretion is pulsatile as has been found in eutherian species. There are significant differences in the pattern of secretion before and after 52 weeks of age; frequency of pulses decreases from 3.6 to 2.6 per 6 hours ($p=0.0872$), pulse magnitude drops from 4.7ng to 1.9ng GH/ml plasma ($p=0.0047$) and basal GH concentration decreases from 3.3ng to 1.0ng GH/ml plasma. Overall a significant decrease in mean GH concentration occurred after 52 weeks of age in both groups ($p=0.0001$ and $p=0.0066$ respectively).

There is an age-dependent relationship between physical growth and plasma GH in the tammar: the greatest proportional physical growth and the highest concentration and pulsatility of GH release occurs prior to 52 weeks of age.

CARDIOVASUCLAR AND RESPIRATORY RESPONSES TO HYPOXIA IN SWIMMING TUNAS

P.G. Bushnell & R.W. Brill*; Dept. of Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 2A9; *National Marine Fisheries Service, Honolulu Laboratory, 2570 Dole Street, Honolulu, Hawaii, U.S.A. 96822-2396.

Tunas have evolved anatomical, physiological and biochemical adaptations that allow them to achieve exceptionally high metabolic rates. As a consequence, the oxygen content of the water is thought to be an important factor in determining the distribution of tunas in the oceans. Since there is little physiological data available to support this idea, experiments were conducted to evaluate the cardiovascular and respiratory responses to hypoxia in these obligate ram ventilators. Restrained and swimming tunas exposed to moderate levels of hypoxia (120-140 mmHg) increased mouth gape (and hence, ventilation volume); and decreased heart rate, cardiac output, and oxygen extraction. The hypoxic bradycardia occurred in swimming tunas in spite of a concomitant increase in swimming speed. The experimental results enabled us to construct a model of oxygen demand and delivery flux in tunas under several conditions. The model suggests that the cardiorespiratory system of tunas permits very high rates of oxygen delivery, not only to support sustained high speed swimming (5 -6 body lengths/sec), but for the rapid repayment of oxygen debt, at low swimming speeds.

THE BLOOD BRAIN BARRIER: A MARSUPIAL ALTERNATIVE TO THE EUTHERIAN SOLUTION ?

Colin J Carati, Grant H White and Bren J Gannon

Dept. of Anatomy and Histology, School of Medicine,
Flinders University, Bedford Park, SA 5042.

The Blood Brain Barrier (BBB) is characterised by capillaries which are relatively impermeable to polar circulating substances. These capillaries have an endothelium with structural characteristics indicative of selective transport function (e.g. tight endothelial cell junctions and an absence of fenestrae). However, the brain does 'communicate' with the blood via 'windows to the brain', known as the circumventricular organs (CVOs). These specialised regions are characterised by a ready permeability of most plasma bourne substances, including hormones, across an endothelium containing large intercellular clefts and fenestrae.

Little is known of the brain microcirculation in marsupials, except that the brain capillaries are arranged in regular hairpin-like loops, rather than the anastomosing networks seen in eutherians. This unusual structure may allow some counter-current exchange across the loop, but the implications of this have not been addressed. Marsupials do possess some CVO's but their microvasculature has not been investigated. This paper describes the distribution of capillary loops and CVO's within the marsupial brain, and their permeability to macromolecules.

Sections of brain from Tammar wallabies (*Macropus eugenii*) and brush-tailed possums (*Trichosurus vulpecula*) were examined by standard light and electron microscopy. The permeability of brain microvessels to macromolecules was examined by fluorescence microscopy after iv injection of Fluorescein Isothiocyanate Bovine Serum Albumin (FITC-bSA, 250 mg/kg, 10 min circulation). FITC-bSA is normally retained within eutherian brain microvessels, except at sites involved in macromolecular flux, i.e. the CVO's.

Capillary loops were found throughout the cortex and cerebellum, and consisted of an arteriolar and venular limb closely apposed within a common basement membrane. Pericytes and cellular processes were invariably seen between the two branches of the loop. The endothelium was non-fenestrated and appeared to have tight junctions. FITC-bSA was contained within the vasculature, as it is in eutherian brain. Thus, much of the marsupial brain microvasculature differed from eutherians only in the fact that it was arranged in loops. The vasculature of the choriod plexus, median emminence and the neural lobe of the pituitary (i.e. CVO's) leaked FITC-bSA and had an appearance similar to their eutherian counterparts, i.e. sinusoidal anastomosing microvessels with numerous fenestrations. However, the pineal and the area postrema (which are CVO's in eutherians) contained capillary loops which did not leak FITC-bSA and which possessed an endothelium typical of BBB microvessels rather than of CVO's.

In conclusion, the marsupial brain has an unusual microvasculature characterised by loops, rather than the anastomosing networks of eutherian brains. The capillary loops are impermeable to FITC-bSA, indicating that they may form a BBB, at least to macromolecules. It is yet to be determined if there is any counter-current exchange between limbs of the loop, and whether this provides an additional functional BBB, e.g.for lipid soluble molecules. The marsupial brain does possess some CVO's which are structurally similar to their eutherian counterparts. However, the area postrema and the pineal have capillary loops and do not leak macromolecules, suggesting that something other than typically eutherian mechanisms facilitate blood-brain flux in these CVO's.

Acknowledgments: This work was supported by the ARC, and significantly enhanced by the contributions of Russ Baudinette.

The Influence of Dietary Phosphate on pH and Buffering Capacity of the Haemolymph of the Cricket *Teleogryllus commodus* . Paul D. Cooper and Roslyn L .Guy. Dept. of Zoology, Aust. Nat. Univ.

Haemolymph pH has been shown to be influenced by feeding, probably as a result of the transfer of acid equivalents from the haemolymph to the crop . To better understand this process, crickets were fed on rye grass grown in artificial medium containing either 0.1 or 0.7 mol L^{-1} phosphate. Haemolymph pH, total CO_2 and phosphate were measured in the two groups of crickets, as well as the phosphate excreted with the faeces. Haemolymph pH was elevated in both groups of crickets immediately after feeding as was total CO_2. Haemolymph phosphate concentration was regulated near 4 mmol L^{-1} under all treatments. This work suggests that the increase in haemolymph pH following feeding may be associated with Na^+/H^+ transport in the digestive system, with bicarbonate being transferred into the haemolymph.

GIANT DINOSAURS WITH LONG NECKS: A MODEL TO DETERMINE THE MOST EFFICIENT LUNGS AND METABOLISM

Christopher B. Daniels and Jonathan Pratt
Department of Physiology, School of Medicine,
The Flinders University of South Australia,
Bedford Park, South Australia 5042.

Breathing through very long necks presents major problems. In particular the increased anatomical dead space reduces the absolute amount of oxygen delivered to the alveoli/breath. To compensate, long necked animals either hyperventilate their bellows type lungs (eg. Giraffes) or have air sac type lungs with a counter current exchange mechanism. The former solution risks hypocaphnia, is energetically expensive, and requires a large lung-atmosphere pressure gradient. Moreover, successive highly negative - highly positive intra-thoracic pressures may seriously impede pulmonary capillary blood flow and affect venous return to the heart. Consequently, long necks together with the bellows type lungs are uncommon amongst both ectoherms and endotherms. Birds frequently couple long necks with a multiple air sac, counter current exchange lung. The large amount of tracheal dead space maybe important in preventing hypocaphnia by acting as a CO_2 reservoir.

Mamenchisaurus huananensis was a 30 tonne herbivorous dinosaur with a 11m long neck. We assumed that this animal could have possessed either a mammalian type bellows lung or a multiple air sac bird type lung and possessed either an endothermic or ectothermic (reptilian) metabolism. We predicted CO_2 production/min for the herbivore using values obtained by "extending" the endothermic and ectothermic allometric regressions for metabolism. With these predictions for alveolar ventilation, we calculated optimal tidal volumes, atmospheric/lung pressure gradients, tracheal radius, total lung volume, breathing frequency and work of breathing for bellows and bird lungs during rest and activity. The only respiratory/metabolic system for M. huananensis for which alveolar ventilation was possible without rupture of pulmonary capillaries or an extremely high energy expenditure was the ectothermic metabolism coupled with an air sac lung. This observation is consistent with our current knowledge of the physiology and phylogeny of these dinosaurs.

Cowlins

THE ROLES OF THE GILLS AND LUNGS IN AIR BREATHING CRABS

By C.A. Farrelly, Sydney University, and P. Greenaway, U.N.S.W.

Land crabs have lungs and these are mostly formed from the lining of the branchial chamber. The type of lung present varies widely between species, ranging from a smooth, simple type to lungs which have various degrees of folding and invagination. The surface area of these different types of lungs varies widely, increasing dramatically in the more complex types, however, the blood/gas diffusion distance across the lung surface is similar in all lung types. The lung vasculature is highly organized with different patterns of perfusion occurring in the different lung types. The O_2 content and PO_2 of pulmonary blood was shown to be significantly above both venous and arterial blood, indicating that the gills of land crabs are less efficient in oxygenating the blood than the lungs. The efficiency of CO_2 excretion across the gills and lungs varied between species but an increasingly important role for the lungs was identified in the more terrestrial species. The fine structure of the gill epithelium in land crabs shows that the gills are well equipped for osmoregulation and this together with the results of blood/gas analysis, suggests that the gills of land crabs are retained primarily for the regulation of salts. The lungs of land crabs are thus the chief site of gas exchange.

Is the size of the joey limited by constraints imposed on the O_2 cascade?

P.B. Frappell
School of Biological Sciences, Flinders University of South Australia

Preconceptions of the joey as either ectothermic or endothermic would prejudice answers to this question, since ectotherms with their lowered metabolic demand would not be expected to place potential limits on the mammalian O_2 cascade. While a possible cause of this lowered metabolism can be attributed to a limitation in respiratory mechanics the advantages of possessing a lowered metabolism in terms of heat balance within the pouch are also evident. The important issue is not whether the animal is ectothermic and therefore expected to have a lowered metabolic rate, but whether the metabolic rate observed is constrained by any limits imposed by steps in the O_2 cascade. Such restrictions would be maximised in the smallest joeys at birth.

Although lack of information on the details of the oxygen cascade means that much of the discussion is speculative, the very small joeys would appear to be approaching the limits of gaseous exchange exhibited by mammals, that of the ventilated lung. However, utilisation of cutaneous gas exchange by the joey could offset any functional or structural constraints imposed by the respiratory system.

PERIODIC AROUSAL DURING HIBERNATION

Fritz Geiser, Zoology, University of New England, Armidale, NSW 2351

Hibernating animals rewarm spontaneously from torpor at intervals of several days or weeks. The duration of torpor bouts differs between species, changes with season, and is temperature dependent. However, it is presently not understood why these energetically expensive arousals from torpor occur at all, nor is it known what regulates their regular occurrence. I propose that the duration of torpor bouts is regulated by (i) the set point for body temperature during torpor, (ii) the body temperature during torpor, and (iii) the metabolic rate during torpor because most of the variation of torpor bout duration can be explained by these variables.

NEUROPEPTIDES IN SYMPATHETIC NEURONS OF LIZARDS: CORRELATIONS WITH BODY SIZE AND THERMOREGULATORY CAPACITY?

Ian
I. L. Gibbins, J. L. Morris and S. Holmgren*
Department of Anatomy and Histology, School of Medicine, Flinders University of South Australia, and * Department of Zoophysiology, University of Goteborg, Sweden.

Neuropeptides act as neurotransmitters in many classes of neurons, including postganglionic autonomic neurons. Neuropeptide Y (NPY) and galanin (GAL) are peptides found in different combinations in sympathetic neurons innervating the cardiovascular system of many vertebrate species. We have investigated the distribution of these peptides in the sympathetic neurons of lizards. GAL was found in the neurons of agamids (9 species), gekkos (2 species) and goannas (V. gouldii) but not skinks (3 species). NPY also was absent from the sympathetic neurons of skinks and gekkos but was present in nearly all sympathetic neurons of the goanna. In agamids, the proportion of sympathetic neurons containing NPY was correlated with the body size of each species ($r=0.72$, $d.f.=27$, $p<0.0001$). These results suggest that NPY is expressed to a greater degree in the sympathetic neurons of lizard species with a well developed capacity to regulate their body temperature via cardiovascular adjustments (goannas, larger dragons). However, the functional role of NPY in these neurons is presently unknown.

Osmotic Regulation in the Anomuran Land Crab *Birgus latro*

Peter Greenaway, Biological Science, UNSW; Harry Taylor, Zoology, Univ of Canterbury; Steve Morris, Biological Science, University of Calgary.

Land crabs produce urine isosmotic with the blood, a feature seemingly incompatible with the need to vary the concentration of excretory fluid in terrestrial environments. The production of excretory fluid is examined in Birgus latro. The crab forms primary urine at a rate twice the drinking rate. This fluid is only slightly modified in the antennal organ and remains isosmotic with the haemolymph. Urine is released into the branchial chambers where it is modified by reabsorption of ions and water, much of the latter by drinking. Crabs on a freshwater drinking regimen reabsorb 99.5% of the filtered ion load although K is usually concentrated in the excreted fluid. The final excretory fluid voided is very dilute and small in volume. Ion reabsorption is by means of ATPases in the gill epithelium and lining of the anterior branchial fold. On saline regimens reabsorption decreases with increasing salinity of water so that most of the ingested ions are excreted. A small retention of ions causes an initial increase in haemolymph osmolality in animals drinking 600 and 1000mosm seawater.

SIXTH COMPARATIVE PHYSIOLOGISTS' MEETING

Adelaide 2nd-3rd December, 1989

INTERACTION OF ORGANIC PHOSPHATES WITH SHEEP HAEMOGLOBIN

R.A.B. Holland, J.F. Hallam, and E.A. Tibben

School of Physiology & Pharmacology,
University of New South Wales,
Kensington, Sydney, NSW 2033,
Australia

It has been accepted that sheep haemoglobins do not significantly interact with 2,3-diphosphoglycerate (DPG). Their intrinsic low O_2 affinity is ascribed to the long side chain of the N-terminal methionine of the β-chains stabilizing the tetramer in the deoxy conformation; and the lack of DPG interaction ascribed to the deletion of the residue at β-2, which shortens the β-chain and also removes the binding site at β-2.

We measured the interaction of solutions of sheep Hbs A, B, and F with 2,3-DPG and with inositol hexaphosphate (IP_6) using a modified HEM-O-SCAN in which the Hb solution is in a thin film. This permitted us to use a concentration of Hb_4 higher than has been used before.

Using a concentration of Hb_4 between 1 and 1.7 mM (normal in red cell about 5 mM) we found that at physiological pH and Cl^- concentration, 2,3-DPG caused a right shift in the O_2Hb equilibrium curves of Hb A, HbB, and HbF (fetal). This effect was found in the absence of CO_2 and at 40 Torr PCO_2. In the presence of CO_2, the factor by which 10 mM 2,3-DPG increased P_{50} was 1.15 for HbA, 1.25 for HbB, and 1.26 for HbF. The rapid increase in red cell 2,3-DPG to 10 mM or more in neonatal lambs, would thus appear to right-shift the O_2Hb equilibrium curve at least partly by direct interaction with the Hb tetramer, and not merely by acting as an additional non-diffusible anion to give increased acidity within the red cells.

The curves were similarly right shifted by IP_6 in lower concentrations. The interaction with IP_6 has not been reported previously.

ADRENERGIC REGULATION OF HEPATIC GLYCOGENOLYSIS IN VERTEBRATES

Peter Janssens, Mandy Giuliano and Jenny Grigg
Department of Zoology, Australian National University, Canberra.

In mammals, hepatic glycogenolysis is controlled by several hormones using cyclicAMP, Ca^{2+} and/or diacylglycerol as intracellular messengers. In teleost fish, lungfish and amphibians, fewer hormones are involved, and cyclicAMP is the sole intra-cellular messenger. This suggests that while ß-adrenergic receptors are common to livers of all vertebrates, α-adrenergic receptors became associated with the liver after amphibians separated from the common vertebrate line. Which adrenergic receptor system is operative in reptiles is clearly of interest.

This study investigates the hormonal control of hepatic glycogenolysis in liver pieces cultured in vitro from a reptile, *Amphibolurus nuchalis*. Adrenaline and glucagon stimulated glycogen breakdown and glucose release, glycogen phosphorylase activity and accumulation of cyclicAMP in the tissue. Neurohypophysial peptides did not affect these parameters. While major control is exerted through cyclicAMP and ß-adrenergic receptors, α-adrenergic receptors also appear to play some role. This is, therefore, the first indication that α-adrenergic receptors may be implicated in the regulation of hepatic glycogenolysis in a non-mammalian vertebrate.

CENTRAL CARDIOVASCULAR DYNAMICS OF ALLIGATORS (ALLIGATOR MISSISSIPPIENSIS)

D.R. Jones, and G. Shelton. Universities of British Columbia, Vancouver, Canada, and East Anglia, Norwich, U.K.

We investigated central cardiovascular dynamics by recording pressures and flows in the heart and aortae of both anaesthetized and unanaesthetized alligators. Right ventricular (RV) pressure was always biphasic and only the first phase appeared in the pulmonary artery (PA). If systematic pressure was low then the second phase of RV contraction opened the left aortic (LAo) valves and anterograde flow occurred in LAo. During left ventricular (LV) systole, anterograde flow occurred in the right aorta (RAo) while flow was retrograde in LAo. When systemic pressure was high, and RV contraction failed to open LAo valves, then a small anterograde flow occurred in LAo during diastole, with a flow pattern similar to that in RAo indicating both aortae were connected through the Foramen of Panizza. In resting animals sustained periods of anterograde LAo flow from the RV occurred in response to even small falls (1-5mm Hg) in systemic pressure. These results show that the potential for R to L blood shunting, due to the unique anatomy, is realized in resting animals.

RESPIRATION IN THE DEVELOPING PORT JACKSON SHARK EMBRYO.

K. Messner, Dept. of Zoology, University of Adelaide.

Port Jackson sharks Heterodontus portusjacksoni are oviparous and deposit 10–12 eggs within a 2 month breeding season beginning in September. The structure of the egg case provides protection for the developing embryo throughout its 12 month incubation. Initially (first 2 months), the egg case is closed. Evidence of high internal P_{O2}, movement of early embryo and early development of external gill filaments, implies that O_2 can diffuse into the case. However diffusion barriers appear large; the case is 1–2 mm thick and the internal albumen is viscous. Later in development, albumen plugs which block respiratory slits, dissolve. Thus, movements by the embryo create a unidirectional flow of water through the slits into the case. Respiration in the developing embryo is aided by changes in the external gill filaments which develop from the gill clefts, and later degenerate as internal gills become more important.

HEAT PRODUCTION IN THE ECHIDNA

Stewart C. Nicol, Niels A. Andersen, and Ute Mesch.
Department of Physiology, University of Tasmania.

The echidna is one of the largest animals to show deep hibernation. but is unusual in that it also shows shallow daily torpor. We have made detailed observations of body temperature of captive echidnas, and measured ventilation and metabolism during normothermia, entry into torpor, deep hibernation, and arousal. Spontaneous activity seems to be maintained as long as T_b does not drop below about 20°C. One of the most striking features of hibernation was the occurrence of apneas of up to one hour, making estimation of metabolic rate extremely difficult. Metabolic rates measured during arousal were greater than any obtained during treadmill activity.

cycles before arousal ⇒ [illegible]
apnea up to 57 min

BLOOD GASES AND BODY TEMPERATURE IN THE ECHIDNA

Niels A. Andersen, Stewart C. Nicol, and Ute Mesch.
Department of Physiology, University of Tasmania,

There is a continuing debate as to whether the low metabolic rates which characterise torpor and hibernation are a physical effect of temperature (Q_{10} effect) or whether there is some additional metabolic inhibition. One suggested inhibitor is respiratory acidosis. We have measured arterial blood gases and pH of echidnas at a variety of body temperatures. Preliminary results indicate that the echidna employs different acid-base strategies above and below 20°C. Above 20°C, i.e. during shallow torpor the echidna, the change in arterial blood pH with changing body temperature, ΔpH/ΔT is about -0.02. Below 20°C, pH appears to kept fairly constant, representing a relative respiratory acidosis.

[illegible] O_2 decline at same T_b [illegible] switch from [illegible] = arousal
to [illegible] of thermoregulatory

STRUCTURAL CHANGES IN THE TAMMAR WALLABY LUNG FROM BIRTH TO ADULT. John H.T. Power School of Medicine, Flinders University, Adelaide, Australia, 5042.

Structural changes in the tammar wallaby lung were examined by fixing wallaby lungs at various ages from birth to adult. At birth the marsupial lung consists of large airsacs with thick septa. Capillaries form small loops around the periphery interdispersed with surfactant secreting alveolar type II cells. At day ten the lung still consists of airsacs but clusters of type II cells appear in the septal tissue which appear to be the sites of future alveoli. Around day 14 there is a period of rapid growth, the airsacs develop a cuboidal epithelium with very little capillary looping. Many type II cells are in contact with areas overlayed by a layer of cells which appear to be differentiating type II cells. By day 31 the cuboidal epithelium has disappeared and the airsac septum is becoming thinner. At day 63 further thinning of the septum has occurred and the lung is more alveolar in appearance. Alveoli continue to become more numerous and the septum continues to thin in a gradual process. The adult structure is not achieved until sometime after 140 days.

EFFECT OF SALT LOADING ON RENAL FUNCTION IN FERAL CHICKENS.

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Renal function was assessed in feral chickens which were infused with a control solution of NaCl (0.75%) followed by a hypertonic NaCl solution (1M). A salt load of 12 mmol/kg body weight was delivered over two 30 minute clearance periods. The infusion of 1M NaCl produced significant increases in plasma osmotic concentration and the concentrations of sodium and chloride. This was associated with increases in urine flow rate, glomerular filtration rate, renal plasma flow and renal blood flow and a decrease in the filtration fraction. Osmolar clearance and the fractional excretion of sodium, chloride and potassium all increased with the infusion of 1M NaCl whereas freewater clearance decreased. The salt load of 12 mmol/kg body weight was not sufficient to cause a decrease in glomerular filtration rate. Rather, it appears to have created a volume expansion leading to an increase in renal blood flow and glomerular filtration rate.

INTER-ATRIAL SEPTAL CHANGES IN THE PERINATAL MARSUPIAL HEART

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In placental mammals, the developing atrium is divided into left and right chambers by a thin sheet-like primary septum containing a secondary foramen on the left atrial side and a thicker, crescentic, secondary septum containing a foramen ovale on the right atrial side. These septa and their respective foramina form a one-way flap-valve structure, the primary septum forming the flap of the valve. Before birth, this arrangement permits most of the well oxygenated placental venous blood, entering the right atrium via the inferior vena cava (IVC), to pass through to the left atrium and via the left ventricle to the aorta. Thus, placental blood bypasses the non-functional lungs prenatally, and is shunted to the systemic circulation. At birth, the placental circulation is occluded, resulting in decreased pressures in the IVC and right atrium. Pulmonary vascular resistance decreases on commencement of ventilation, resulting in a marked increase in pulmonary blood flow. Consequently, left atrial pressure rises to exceed right atrial pressure for the first time; hence the primary septum is pushed against the secondary septum and blood shunting from right to left atrium ceases within minutes of birth.

The interatrial septum in the tammar wallaby (*Macropus eugenii*), was assessed in prenates and neonates (up to four days postnatally). Specimens were serial sectioned, sections stained, and observations carried out using a video light microscope and video printer; a 3-D reconstruction computer program was used to view the septal structure. The intact septum was also observed in other specimens by scanning electron microscopy.

In marsupials, which are born at a very early stage of development, only a primary septum separates left and right atrial chambers at birth. This septum is extensively fenestrated and clearly could permit significant right to left shunting before birth. The secondary septum is only a rudimentary structure which does not overlap the fenestrae in the primary septum, and so no valving of the interatrial communication is possible following birth. The fenestrae in the primary septum close by tissue proliferation, around their margins, over 4 to 5 days postnatally.

Thus, in contrast to placental mammals, in which the right to left interatrial shunt ceases at birth, in marsupials the interatrial communication is potentially bi-directional and persists for up to five days after birth. The fenestrae may by important for the marsupial neonate, as exact matching of left and right ventricular outputs, perhaps beyond the control system capabilities of the newborn joey, is not mandatory with persisting interatrial fenestrae. Actual atrial pressures and interatrial flows remain, however, unknown. The incomplete development of the secondary septum in marsupials is intriguing; a provocative interpretation could be that the "precocious" marsupial birth intervenes during cardiac development, before the secondary septum is complete.

RESPIRATION IN TERRESTRIAL FROG EGGS. R.S. Seymour, Dept. of Zoology, University of Adelaide.

During the development of Pseudophryne bibroni, the eggs absorb water from the substrate, through the jelly capsule, and into the perivitelline fluid where the embryo respires. Water absorption increases the diameter, and reduces the thickness, of the capsule wall, both effects joining to increase the diffusive O_2 conductance (G_{O_2}) of the capsule. At 12°C, changes in G_{O_2} occur in parallel with increasing $\dot{V}_{O_2}$ such that the internal P_{O_2} remains high and constant throughout incubation. However, G_{O_2} does not appear to be adaptively regulated, because incubation at different temperatures or ambient P_{O_2} levels do not alter the course of G_{O_2} changes. Consequently the P_{O_2} in the perivitelline fluid is also unregulated and declines at higher temperatures or lower ambient P_{O_2}. Above 17°C, for example, $\dot{V}_{O_2}$ becomes diffusion limited. The embryo is apparently developmentally programmed to secrete specific substances that modify capsule morphology at specific stages of development, and cannot respond to environmental conditions by altering the time-course of secretion.

Phenotypic inhibition of the renin-angiotensin system (RAS) in mice preceded extensive expression of salivary gland renin.

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Summary:

The RAS in sub-genus *Mus* displays unique features including duplication of the renin gene in most strains, strong expression of the second gene in submandibular gland of males and inhibited responses to injected renin. Our findings indicate that this inhibition results from a paucity of renin substrate and is consistent with first order kinetics. We find substrate paucity to be a feature of both sexes and all sub-species and strains of *Mus* irrespective of gene duplication. Attempts to increase the level of substrate in blood by intravenous injection caused marked increases in blood pressure in *Mus* suggesting that substrate paucity was a phenotype prerequisite for successful emergence of enhanced renin expression in salivary gland. These phenomena together with 'lethal factor' transmitted in saliva by biting appear to be linked in an evolutionary sequence that has provided a major selective advantage for *Mus* and influenced the ecology and evolution of rodents.

Release of the embryo from diapause near the end of pouch suckling in Bettongia penicillata

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Sharman's studies on the red kangaroo showed that although the corpus luteum of lactation was held in quiescence by the suckling stimulus for most of pouch suckling, corpus luteum growth and embryonic development do occur concurrently with pouch suckling in the 3-4 weeks before the pouch young emerges permanently. Development of the corpus luteum was believed to occur because intermittent suckling by the large young led to a fall in the hormone inhibiting the corpus luteum (Sharman 1965).

The brushtailed bettong, B. penicillata, breeds continuously and, similarly to the situation in the red kangaroo, quiescent corpus luteum and diapause embryo resume development while the pouch young suckles within the pouch. The effect on this redevelopment of sucking by the young in the pouch has been investigated by a) exchanging young of different ages between pouches, and b) giving postpartum bettongs a second neonate and allowing two young to develop in the one pouch.

Neither treatment affected the time of development of the corpus luteum and embryo.

Sharman, G.B. (1965). Zeitschrift fur Saugetierkunde 30 10-20.

Patterns of metabolism in embryonic reptiles

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Three basic patterns of the rate of change of $\dot{V}_{O_2}$ during incubation occur in embryonic reptiles. The rate may:
1. increase throughout incubation (in snakes),
2. decline late in incubation (in many turtles and some lizards),
3. reach a peak followed by a significant fall prior to hatching (in some turtles and crocodilians).

I have postulated that the peaked pattern is a mechanism that facilitates synchronous hatching. This theory assumes that:

(a) developmental asynchrony occurs in some species,
(b) synchronous hatching is important, and
(c) there is communication between eggs.

There is evidence for (a) from temperature data in nests at different depths and (b) has a little supporting data. Because vocal commmunication occurs in crocodilians, I tested the theory using American alligators by placing a group of young eggs with older eggs. The results support the hypothesis: eggs incubated in the presence of older eggs hatch significantly earlier than controls incubated in isolation.

TITLE REPIRATION AND ACID-BASE BALANCE DURING EMERSION IN THE MUDCRAB, *Scylla serrata*.

AUTHOR Glenn Varley, Biological Science, UNSW

ABSTRACT

The effect of emersion periods of up to 7 days on blood gas and acid-base balance was studied. Mean arterial and venous haemolymph pCO_2 rose in the first six hours of emersion. Total carbon dioxide content rose from 7 mmol/l in immersed crabs to 20 mmol/l after 7 days emersion. Mean arterial pO_2 and total oxygen fell within 1 hour of emersion but were restored after 7 days emersion. Total oxygen content decreased drastically when crabs were re-immersed into aerated sea water. A respiratory acidosis suffered during the initial period of emersion was compensated for after 3 days emersion by elevation of bicarbonate levels inferring that an increase in strong ion difference had also occurred. The increased strong ion difference could not be accounted for by increased concentrations of lactic acid or calcium ions which showed no significant changes. On re-immersion the animals showed drastic changes in acid-base balance which possibly also contributed to the abovementioned rapid decline in oxygen carrying capacity.

Late addition

CHEMICAL CODING OF PARASYMPATHETIC NEURONS OF THE TOAD, *BUFO MARINUS*

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In recent years it has been established that neurons contain and can release more than one chemical substance. The association of a particular array of colocalized transmitters with a discrete population of functionally similar neurons, a phenomenon refered to as chemical coding, has been reported in a variety of mammals (Furness et al., 1989). We have examined the chemical content of a group of cranial and sacral parasympathetic neurons of an amphibian, the cane toad, in order to determine whether toad parasympathetic neurons, like those in mammals show "chemical coding".

Tissues were obtained from animals over-anaesthetized by immersion in 0.5% tricaine methanesulphonate (MS222, Sandoz). Dual-labelling immunohistochemical techniques were used to determine the coexistence of neuropeptides using antibodies for calcitonin gene-related peptide (CGRP), galanin (GAL), 5-hydroxytryptamine (5-HT), somatostatin (SOM), substance P (SP) and vasoactive intestinal polypeptide (VIP). Due to the lack of a suitable histochemical marker for acetylcholine (ACh), *in vitro* physiological experiments were used to determine the presence or absence of cholinergic neurons.

Based on their chemical content determined by a combination of physiological and immunohistochemical techniques, post-ganglionic parasympathetic neurons can be divided into two distinct groups. First, cholinergic parasympathetic postganglionic nerves innervating the lung, pulmonary artery, urinary bladder and rectum contain immunoreactivity (IR) to two peptides, GAL and SOM; those in the rectum were found to contain, the indoleamine 5-HT as well. Second, non-cholinergic parasympathetic postganglionic nerves innervating the lingual vasculature, pulmonary artery, lung and rectum contain IR to VIP; those innervating the lingual vasculature also contain GAL-IR.

Preganglionic parasympathetic fibres in the vagus nerve forming synaptic boutons on postganglionic nerve cell bodies, contain colocalized CGRP and SP: synaptic boutons containing CGRP- and SP-IR are associated with both ACh/GAL/SOM and VIP/- cell bodies. Preganglionic parasympathetic nerves forming boutons on GAL/SOM nerve cell bodies in the rectum and urinary bladder contain CGRP and VIP without SP.

Furness, J.B., Morris, J.L, Gibbins, I.L. & Costa, M. (1989) Annu. Rev. Pharmacol. Toxicol., 29, 289-306.